(54) Title: PEPTIDE-BASED NUCLEIC ACID MIMICS (PENAMs)

(57) Abstract

The present invention provides novel nucleic acid mimics (termed "PENAMs") comprising a peptidic backbone and nucleotidic sidechains; the sidechains being oriented in such a way that the PENAM is homomorphous to target nucleic acids with which it can effectively hydrogen bond. Homomorphism is achieved by the incorporation of unusual stereochemical centers, including D-chiral centers and quasi-chiral centers, into the peptidic backbone. The PENAMs are useful for targeting nucleic acid sequences in order to modulate their activity in an "antisense" manner. Targeting can also be used to detect, isolate or modify target nucleic acids.

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PEPTIDE-BASED NUCLEIC ACID MIMICS (PENAMs)

Field of the Invention

The invention generally relates to compounds that
10 are capable of binding to nucleic acids in a sequence-specific manner. More specifically, the invention provides novel chimeric molecules having a backbone that resembles a polypeptide but side chains that resemble the base portions of nucleic acids. The
15 invention also relates to the construction of such chimeric molecules; and to their use in targeting complementary nucleic acids.

Background

20 Nucleic acids can be targeted by virtue of the ability of "antisense" nucleotidic bases to engage in hydrogen bonding with complementary bases of a target nucleic acid, thereby modulating expression of the target. The use of sequence-specific recognition of
25 pathogenic genes to regulate or inhibit their expression in an antisense manner, using short synthetic complementary oligo(deoxy)nucleotides ("ODNs") and their analogs has been an attractive strategy for rational drug design [1, 2]. [Throughout this document, arabic numbers
30 in square brackets refer to one or more references cited in the the list of References below.]

A simple illustration of such a technique would be the administration of a DNA oligomer complementary to a target mRNA that encodes a protein necessary for the
35 progression of infection of an agent such as a virus. In such a case, the administered DNA might bind to the target mRNA transcript, resulting in translation arrest,

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thereby blocking the synthesis of a protein critical to infection. Hybridization to a nascent mRNA may lead to premature termination of transcription. It is also possible to target double-stranded DNA using an
5   appropriate oligomer capable of engaging in the formation of a triple helix via insertion of the oligomer into the major groove of the double-helical DNA.

General approaches to the construction and modification of oligomers useful in antisense therapy
10   have been described in a number of reviews, see, e.g., Miller & Ts'o [1], Matteucci & Bischofberger [2], Cohen & Ghosh [3, 4], Helene & Toulme [5], Stein [6, 7], Uhlmann & Peyman [8], and the papers reviewed therein. In addition, a publicly available bibliography of
15   citations relating to antisense oligonucleotides is maintained by Dr. Leo Lee at the Frederick Cancer Research Facility in Frederick, Maryland, which references are hereby incorporated herein.

Although there have been some promising results from
20   the use of antisense ODNs in cell culture, suggesting the potential of this methodology for inhibiting gene expression, ODNs are subject to two significant limitations: (i) relatively poor transport across cellular membranes and (ii) sensitivity to cellular
25   nucleases [4].

There have been a number of attempts to circumvent these problems by modifying the oligomers. For example, since nucleases are known to attack the phosphodiester linkage, a number of modified oligonucleotides have been
30   prepared which contain alternate linkages, such as methylphosphonates (wherein a phosphorous-linked oxygen has been replaced with a methyl group), phosphorothioates (wherein a phosphorous-linked oxygen has been replaced with a sulfur group), and various amidates (wherein NH_2 or
35   an organic amine replaces one of these oxygen atoms); see e.g., the work primarily reviewed by Uhlmann & Peyman [8] and others [9-14].

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Other efforts have been focused on changing or replacing phosphodiester groups or other parts of the sugar phosphate backbone by variety of linkages which include, for example: carbamate derivatives and
5   morpholine derived carbamate derivatives [15, 16]; polyvinylic derivatives [17, 18]; polyphosphate derivatives [19]; polylysine derivatives and polyethylene amine derivatives [20]; peptidic derivatives [21-33]; nylon-based derivatives [34, 35]; and polyamide
10   derivatives [36-42].

In some of these cases the modified oligomers were capable of hybridizing to the target nucleic acid, but exhibited shortcomings related to stability, sensitivity to degradative enzymes and/or cellular uptake. In some
15   other cases, the modified oligomers were relatively stable, and fairly resistant to degradative enzymes (due to the unusual linkages), but hybridization between these modified oligomers and their nucleic acid targets was sub-optimal. (As described below, we believe that some
20   of these shortcomings may be due to the electrostatic and/or spatial properties of these oligomers.)

Accordingly, there remains a need for molecules that are: (i) relatively easily taken up and transported by cells; (ii) relatively stable and resistant to cellular
25   degradative enzymes; and (iii) effective at binding to complementary nucleic acids.

Summary of the Invention

It is a primary object of the present invention to
30   provide a molecule that is able to function like a nucleic acid in antisense targeting but which overcomes certain of the disadvantages associated with the use of oligonucleotides (ODNs) for antisense targeting.

The present invention provides a chimeric molecule
35   referred to as a "peptide-based nucleic acid mimic" (or "PENAM"). These chimeric molecules have a peptide-like backbone with side chains that contain bases resembling

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those normally found in nucleic acids. The bases on the
nucleic side chains of the PENAM allow the molecule to
engage in hydrogen bonding with a target nucleic acid
having a substantially complementary sequence of bases;
5   or to engage in triple helix formation, as described
below.

The chimeric PENAMs of the present invention also
have an unusual stereochemical composition that
facilitates binding to the target nucleic acid. In
10   particular, the PENAMs of the present invention have a
peptidic backbone that incorporates unusual chiral
centers (including D-chiral centers and quasi-chiral
centers) that can be used to orient the nucleic side
chains in such a way that the nucleotidic bases are
15   spatially homomorphic to bases in targeted nucleic acids.
The ability to enhance binding by spatial homomorphism is
especially significant given that hydrogen bonding
interactions between biomolecules typically depend on an
aggregation of many relatively weak bonds. The PENAMs
20   are also much less susceptible to electrostatic charge
repulsion (because of the replacement of the normally
charged backbone). Also, by virtue of their unusual
structural and stereochemical features, the PENAMs of the
present invention are resistant to degradative enzymes
25   that are expected to be present in most biological
systems. In particular, the PENAMs do not possess the
phosphodiester backbone which is the standard target of
the nucleases. Moreover, the peptidic backbone is unlike
that of naturally occurring peptides because of the
30   presence of unusual chiral centers including D-chiral
centers and/or quasi-chiral centers.

In addition, the peptidic backbone is assembled from
monomers (termed "NuAA monomers", described below) which
can themselves be conveniently linked using peptide
35   synthetic techniques. These building block NuAA monomers
thus allow for the rapid production of a variety of
"antisense" oligomers (exhibiting differing sequences for

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targeting particular complementary nucleic acids), via an automated peptide synthesizer.

Thus, one aspect of the present invention is a novel chimeric oligomer having bases that resemble those in 5 ODNs, but in which the entire ribose- or deoxyribose-phosphate backbone has been replaced with a peptidic backbone that has been designed to incorporate unusual chiral centers including D-chiral centers and/or quasi-chiral centers. The resulting peptidic backbone is 10 capable of orienting the side chains such that the nucleic acid mimic can effectively bind to a complementary target nucleic acid. These novel chimeras, termed "peptide-based nucleic acid mimics" (or "PENAMs") are stable under physiological conditions and resistant 15 to degradative enzymes, and are electrostatically and spatially designed for enhanced transport across cellular membranes and effective binding to target nucleic acids.

One aspect of the invention is a composition useful in targeting a nucleic acid comprising a 20 stereochemically-selected population of peptide-based nucleic acid mimics (PENAMs) each member of which comprises a sequence of at least about 4 NuAA monomers, wherein at least one of said PENAMs is a homomorphically-preferred PENAM comprising a D-chiral center or a quasi-25 chiral and wherein said homomorphically-preferred PENAM makes up at least about 10% of the stereochemically-selected population of PENAMs. The NuAA monomers are each of the following formula:
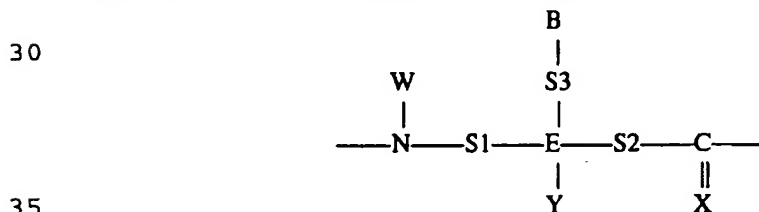
30
$$
\begin{array}{ccc}
 & & B \\
 & & | \\
 & W & S3 \\
 & | & | \\
—N—S1—E—S2—C— \\
 & | & \| \\
35 & Y & X
\end{array}
$$

wherein:

E is carbon (C) or nitrogen (N);

W is hydrogen or a spacer group;

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Y is hydrogen or a spacer group (when E is carbon),
or Y is a lone pair of electrons (when E is nitrogen);

S1 is a bond or a first spacer group;

S2 is a bond or a second spacer group;

5   S3 is a bond or a third spacer group;

X is oxygen (O) or sulfur (S);

B is a base segment comprising a nucleotidic base or
an analog thereof; and

N is nitrogen.

10   Preferably, the homomorphically-preferred PENAM
makes up at least about 30% of the stereochemically-
selected population of PENAMs; more preferably at least
about 50%; still more preferably at least about 80%.

Another aspect of the invention is free NuAA monomer

15 that is a precursor of a NuAA monomeric subunit
comprising a quasi-chiral center wherein said NuAA
monomeric subunit comprising a quasi-chiral center is of
the following formula:

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\begin{array}{ccccc}
 & & \overset{\textstyle B}{\overset{\textstyle |}{}} & & \\
 & \overset{\textstyle W}{\overset{\textstyle |}{}} & \overset{\textstyle S3}{\overset{\textstyle |}{}} & & \\
—\!\!—N\!\!—\!\!—S1\!\!—\!\!—E\!\!—\!\!—S2\!\!—\!\!—C\!\!—\!\!— \\
 & & \underset{\textstyle |}{\underset{\textstyle Y}{}} & & \underset{\textstyle \|}{\underset{\textstyle X}{}}
\end{array}
$$

25   wherein:

E is nitrogen (N);

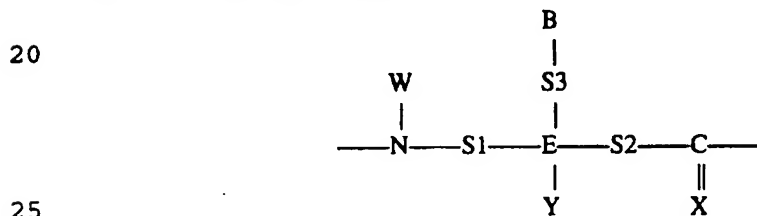W is hydrogen or a spacer group;

30   Y is hydrogen or a spacer group (when E is carbon),
or Y is a lone pair of electrons (when E is nitrogen);

S1 is a bond or a first spacer group;

S2 is a bond or a second spacer group;

S3 is a bond or a third spacer group;

35   X is oxygen (O) or sulfur (S);

B is a base segment comprising a nucleotidic base or
an analog thereof; and

N is nitrogen;

wherein said free NuAA monomer is of the same
formula except that it has an amine group or a protected
amine group at its N-terminus and it has a carboxyl group
or a protected carboxyl group at its C-terminus.

5   Another aspect of the invention is a free NuAA
monomer that is a precursor of a NuAA monomeric subunit
comprising a D-chiral center wherein said NuAA monomeric
subunit comprising a D-chiral center is of the following
formula:

$$
\begin{array}{ccccc}
& & B & & \\
& & | & & \\
& W & S3 & & \\
& | & | & & \\
\!-\!\!-\!\!N\!-\!\!-\!S1\!-\!\!-\!E\!-\!\!-\!S2\!-\!\!-\!C\!-\!\!- \\
& & | & & \| \\
& & Y & & X
\end{array}
$$
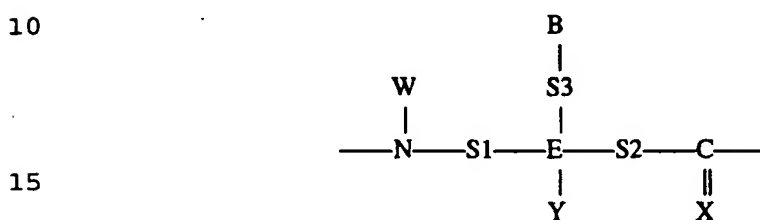
wherein:

E is carbon (C);

20   W is hydrogen or a spacer group;

Y is hydrogen or a spacer group;

S1 is a bond or a first spacer group;

S2 is a bond or a second spacer group;

S3 is a third spacer group with a backbone of at

25   least two atoms;

X is oxygen (O) or sulfur (S);

B is a base segment comprising a nucleotidic base or
an analog thereof; and

N is nitrogen;

30   and wherein at least one of S1 and S2 is a spacer
group, and wherein S3 is a spacer group with a backbone
of at least two atoms;

wherein said free NuAA monomer is of the same
formula except that it has an amine group or a protected

35   amine group at its N-terminus and it has a carboxyl group
or a protected carboxyl group at its C-terminus.

Another aspect of the invention is a method of
preparing a PENAM composition for targeting a target
nucleic acid comprising: (a) providing at least about 4

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NuAA monomers, preferably at least about 6 NuAA monomers, wherein at least one of said NuAA monomers comprises a D-chiral center or a quasi-chiral center; and (b) synthesizing an oligomer of the monomers such that the

5 arrangement of bases in the oligomer is substantially complementary to a sequence of nucleotidic bases in a portion of the target nucleic acid. Preferably step (a) involves providing at least about 6 NuAA monomers, more preferably at least about 10 NuAA monomers, still more

10 preferably at least about 14 NuAA monomers. The NuAA monomers can be linked to each other directly forming peptide bonds, or indirectly via an intervening residue such as an amino acid residue.

Another aspect of the invention is a method of

15 targeting a target nucleic acid comprising: (a) providing a PENAM composition; and (b) contacting said PENAM composition with the target nucleic acid.

Another aspect of the invention is a method of modulating a target nucleic acid in an antisense manner

20 comprising: (a) providing a PENAM composition wherein the sequence of bases in said NuAA monomers is substantially complementary to a sequence of bases in the target nucleic acid; and (b) contacting said PENAM composition with the target nucleic acid.

25 Another aspect of the invention is a method of modifying a target nucleic acid comprising: (a) providing a PENAM composition wherein said homomorphically-preferred PENAM further comprises a target modifying group; and (b) contacting said PENAM composition with the

30 target nucleic acid.

Another aspect of the invention is a method of detecting a target nucleic acid comprising: (a) providing a PENAM composition wherein the sequence of bases in said NuAA monomers is substantially complementary to a

35 sequence of bases in the target nucleic acid; and (b) contacting said PENAM composition with the target nucleic acid; and (c) detecting a target complex comprising said

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nucleic acid mimic and said target nucleic acid or
detecting a modification in the target nucleic acid.

Another aspect of the invention is a method of
isolating a target nucleic acid comprising: (a) providing
5   a PENAM composition wherein the sequence of bases in said
NuAA monomers is substantially complementary to a
sequence of bases in the target nucleic acid; and (b)
contacting said PENAM composition with the target nucleic
acid; and (c) isolating PENAMs bound to said target
10 nucleic acid or isolating a modified target nucleic acid.

Brief Description of the Drawings
        Figure 1 is a comparison of monomers in
oligo(deoxy)nucleotides   (such   as   DNA   and   RNA,
15 collectively referred to as "ODNs"), and the NuAA
monomers that form the peptide nucleic acid mimics
(PENAMs) of the present invention.
        Figure 2 is an illustrative example of molecular
modelling of an (L)-PENAM (Version I, constructed from L-
20 NuAA monomers) and a (D/L)-PENAM (Version II, constructed
from alternating D- and L-NuAA monomers), shown in
beta-sheet conformation in both a "side view" (Figure 2a)
and a "top view" (Figure 2b).
        Figure 3 is an illustrative example of molecular
25 modelling comparisons of various PENAMs (differing in the
length of their "S2" spacer group), each bound to a
complementary strand of target DNA. The S2 spacer group
positions are occupied by bonds (Figure 3a), methylene
groups (Figure 3b), ethylene groups (Figure 3c), or
30 propylene groups (Figure 3d); as described in Example 2
below. Figure 3e is a corresponding model illustrating
the interaction between two complementary DNA strands.
        Figures 4a and 4b are illustrative examples of the
retrosynthesis of PENAMs and NuAA monomers from
35 α-amino-α-carboxylic acids.

Figure 5 is an illustrative example of the synthesis
of a NuAA monomer and a PENAM starting from
(D/L)-methionine.

Figure 6 illustrates a synthetic strategy for
5 preparing NuAA monomers containing various nucleic bases
(A, T, G, C, U).

Figure 7 is an illustrative example of the
retrosynthesis of PENAMs and NuAA monomers from α-amino-
ω, α-dicarboxylic acids.

10 Figure 8 is an illustrative example of the
retrosynthesis of aza-NuAA monomers from aza-amino acids.

Detailed Description of the Invention

15 Definitions

The following definitions are intended to
supplement, not to replace, the detailed descriptions of
these and other aspects of the invention which are
described herein.

20 A "peptide-based nucleic acid mimic" (or "PENAM",
sometimes referred to as a "peptide nucleic acid mimic"
or "nucleic acid mimic"), refers to a molecule having a
peptidic backbone with side chains having nucleotidic
bases that are capable of engaging in hydrogen bonding

25 with a nucleic acid having a complementary sequence of
bases. The PENAMs of the present invention are assembled
from NuAA monomers, but may also contain other chemical
groups such as "target modifying groups", natural amino
acids or peptides, and other groups as described below.

30 Such additional groups may be located within an
individual NuAA monomer, or may occupy positions either
between NuAA monomers or outside of a string of NuAA
monomers. The term "monomer" is used to refer to
individual molecules that can be incorporated by

35 polymerization into larger molecules; and is also used to
refer to the corresponding monomeric subunits (i.e. after
incorporation into the larger molecule).

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The term "complementary" indicates that a particular sequence of bases is able to pair (as in Watson-Crick base-pairing) with corresponding bases in a given target sequence. The term "substantially complementary" indicates that at least about 80% of the bases in a particular sequence are able to engage in base-pairing with corresponding bases of the target sequence. The term "partially complementary" indicates that at least about 60% of the bases in a particular sequence are able to engage in base-pairing with corresponding bases of the target sequence.

A "target nucleic acid" refers to a nucleic acid comprising a sequence of bases that is at least partially complementary to a sequence of base groups on a targeting PENAM.

The "peptidic backbone" refers to the portion of a PENAM that comprises peptide bonds derived from the aminoacyl portions of the NuAA monomers and/or intervening aminoacyl monomers such as natural or unnatural amino acids.

The "nucleic side chains" refer to portions of the PENAM that comprise nucleotidic bases which are capable of engaging in hydrogen bonding with corresponding bases on a target nucleic acid.

A "nucleic aminoacyl monomer" (or "nucleic aminoacyl monomeric subunit") refers to a monomeric subunit of the following formula:
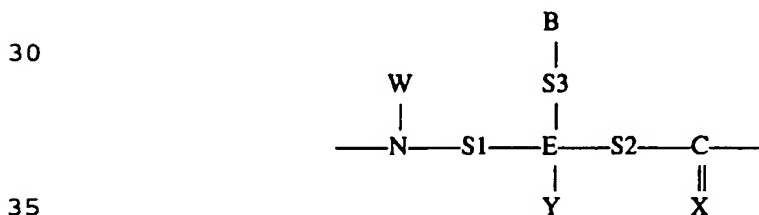
$$
\begin{array}{ccc}
 & & B \\
 & & | \\
 & W & S3 \\
 & | & | \\
 —N— & S1— & E——S2——C— \\
 & & | \quad\quad \| \\
 & & Y \quad\quad X
\end{array}
$$

wherein:

E is carbon (C) or nitrogen (N);

W is hydrogen or a spacer group;

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Y is hydrogen or a spacer group (when E is carbon),
or a lone pair of electrons (when E is nitrogen);

S1 is a bond or a first spacer group;

S2 is a bond or a second spacer group;

5   S3 is a bond or a third spacer group;

X is oxygen (O) or sulfur (S);

B is a base segment comprising a nucleotidic base or
an analog thereof; and

N is nitrogen.

10   A "free nucleic amino acid monomer" (or "free NuAA
monomer") refers to a precursor of a nucleic aminoacyl
monomer. The precursor monomer is essentially of the
same formula as shown above except that the N-terminus
(i.e. the N with a free bond in the formula) is in the
15   form of an amine or protected amine (e.g. a carbamate);
and the C-terminus (i.e. the C with a free bond in the
formula) is in the form of a carboxylic acid or, e.g., an
ester.

"NuAA monomer" is used to refer to both free NuAA
20   monomers and to corresponding NuAA monomeric subunits
after incorporation into a larger molecule.

An "aza-NuAA monomer" refers to a NuAA monomer of
the formula shown above in which E is nitrogen. The
nitrogen at position E forms a "quasi-chiral center" as
25   discussed herein.

An "L-NuAA monomer" refers to a NuAA monomer in
which E is carbon and is a chiral center that is in a
stereochemical configuration corresponding to that of an
analogous L-amino acid (i.e., an L-amino acid having an
30   R group in place of S3-B and having carbonyl and amide
groups oriented in the directions as shown in the formula
above).

A "D-NuAA monomer" refers to a NuAA monomer in which
E is carbon and is a chiral center that is in a
35   stereochemical configuration corresponding to that of an
analogous D-amino acid (i.e., a D-amino acid having an R
group in place of S3-B and having carbonyl and amide

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groups oriented in the directions as shown in the formula above). The carbon at position E in a D-NuAA monomer forms a "D-chiral center" as discussed herein.

An "aminoacyl monomer" (or "AA monomer") refers to
5   a structure that resembles a NuAA monomer as shown in the formula above, but lacks the base segment at position B. Thus, AA monomers are derived from amino acids such as a natural or unnatural amino acids. Such AA monomers can be incorporated into PENAMs and can (either singly or in
10 strings) occupy positions between NuAA monomers. Such AA monomers can, like NuAA monomers, comprise D-, L- and/or quasi-chiral centers.

An "aza-aminoacyl monomer" (or "aza-AA monomer") refers to an analog of an AA monomer in which the chiral
15 center (i.e. the carbon at position E) is replaced with a quasi-chiral center (i.e. a nitrogen at position E).

A "stereochemically-selected population of PENAMs" refers to a population of PENAMs each member of which comprises a sequence of at least about 4 NuAA monomers,
20 wherein at least one of said PENAMs, denoted a "stereochemically-prevalent PENAM" makes up at least about 10% of said population of PENAMs, preferably at least about 30%, more preferably at least about 50%, still more preferably at least about 80%. Generally, the
25 remaining PENAMs in the stereochemically-selected population will be diastereomers of the stereochemically-prevalent PENAM(s); although the population may also include PENAMs of other lengths and/or structure. Stereochemically-selected populations of PENAMs can be
30 most conveniently prepared by utilizing enantiomerically pure starting reagents (preferably at least about 90% enantiomeric excess, more preferably at least 95%, still more preferably at least about 99%), such as D- or L-amino acids, as described herein. Alternatively, one
35 could resolve selected stereochemical intermediates or products, or employ stereo-selective synthetic techniques.

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A "homomorphically-preferred" PENAM refers to a PENAM that exhibits structural and stereochemical properties that facilitate binding between a sequence of nucleotidic bases in the PENAM and a substantially

5   complementary sequence of bases on a target nucleic acid. Homomorphically-preferred PENAMs of the present invention comprise at least one D-chiral or quasi-chiral center. Homomorphically-preferred PENAMs can comprise various combinations of monomers including, e.g., NuAA monomers

10 and AA monomers (both of which can contain D- and/or L-chiral centers); and aza-NuAA monomers and aza-AA monomers (both of which contain quasi-chiral centers).

The term "flanked" or "flanking" refers to the two positions in the peptidic backbone immediately adjacent

15 to a particular NuAA monomer.

A "spacer group" refers to a relatively small chemical group, or series of small chemical groups, that may be included in the peptidic backbone or nucleic side chains either between adjacent atoms (i.e., at positions

20 S1, S2, and/or S3), or dependent from atoms (i.e. at W and/or Y) in the formula shown above; and that is unlikely to inhibit hydrogen bonding between the targeting PENAM and the target nucleic acid. These spacer groups contain a backbone of 1-6 atoms (not

25 counting hydrogen atoms), preferably selected from carbon, nitrogen, oxygen, and sulfur. Typically, such spacer groups are substituted or unsubstituted alkyl, alkenyl, alkynyl groups. However spacer groups can also comprise for example: carbonyl (C=O), thiocarbonyl (C=S),

30 amine (NH), substituted amine (NR), amide {C(=O)NH}, substituted amide {C(=O)NR}, carbamate {NHC(=O)O}, urea {NHC(=O)NH}, thioamide {C(=S)NH}, substituted thioamide {C(=S)NR}, hydrazine (NH-NH), substituted hydrazine {N(R)-N(R)}, ether (C-O-C), thioether (C-S-C), disulfide

35 (S-S), sulphone {S(=O)} and sulphoxide (SO2) groups. The spacer group backbone can also be substituted with one or more small chemical groups, for example, small chain

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alk(ane, ene, yne)s, hydroxyl (OH), alkoxyl (OR), ketone
(COR), aldehyde (CHO), thiol (SH), amine (NH2), and
halogen (F, Br, Cl) groups. A single NuAA monomer may
contain more than one spacer group (at W, Y, S1, S2 and
5 S3 for example) and these need not be identical to each
other. Currently preferred spacer groups are selected
from methylene, ethylene and propylene groups.

A "base segment" refers to a chemical group
comprising the base portion of a nucleotidic base or an
10 analog thereof, as described below.

A "nucleotidic base" or "nucleic base" refers to a
nitrogenous heterocyclic group typically found in nucleic
acids (such as the purine bases adenine and guanine, or
the pyrimidine bases cytosine, thymine and uracil) or an
15 analog thereof. An "analog" of a nucleotidic base
refers, for example, to an analog of a purine base in
which the ring substituents are other than those found in
adenine or guanine, or an analog of a pyrimidine base in
which the ring substituents are other than those found in
20 uracil, thymine and cytosine. Examples of such analogs
are described below and in the art.

A "target modifying group" refers to a chemical
group on a PENAM that is capable, after the PENAM has
hydrogen bonded to a target nucleic acid, of modifying
25 the target nucleic acid. Such modifying groups include
for example groups which label, reconform, cleave,
covalently bind or intercalate into nucleic acids.
Examples of such target modifying groups are described
below.

30   A nucleic acid "labeling group" refers to a group on
a PENAM which, as a result of the interaction between the
mimic and its target, facilitates labeling and thus
detection of the target nucleic acid. Examples of such
labeling groups are described below.

35   A nucleic acid "intercalating group" refers to a
group on a PENAM which, as a result of the interaction
between the mimic and its target, intercalates into

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double helical target nucleic acid. Examples of such intercalating groups are described below.

A nucleic acid "cleaving group" refers to a group on a PENAM which, as a result of the interaction between the
5 mimic and its target, facilitates the cleavage of the target nucleic acid. Examples of such cleaving groups are described below.

The phrase "modulating in an antisense manner" refers to modulating the expression and/or activity of a
10 nucleic acid via hydrogen bonding interactions analogous to those described in systems involving antisense oligonucleotides.

The term "homomorphic" (or "homomorphous") refers to the stereochemical fit between a sequence of bases in the
15 targeting portion of a PENAM and a target nucleic acid wherein at least about 1/2 of the bases of the targeting portion of the mimic are positioned to allow hydrogen bonding with complementary bases on the target nucleic acid. Preferably at least about 2/3 of the bases are so
20 positioned; more preferably at least about 3/4 of the bases are so positioned; most preferably all of the bases are so positioned.

All publications cited herein, including scientific
25 papers, published patent applications and issued patents, are incorporated herein by reference in their entirety.

1.   Nucleic Aminoacyl Monomers (NuAA Monomers)

The peptide-based nucleic acid mimics ("PENAMs") of
30 the present invention comprise a sequence of nucleic aminoacyl monomers ("NuAA monomers"). NuAA monomers are chimeric molecules and/or monomeric subunits of the following formula:

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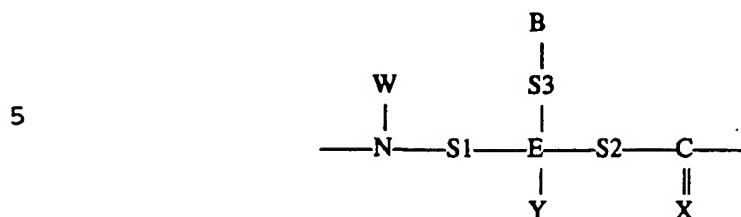
$$
\begin{array}{c}
\text{B} \\
|\\
\text{W}\qquad\text{S3}\\
|\qquad\quad|\\
\text{——N——S1——E——S2——C——}\\
|\qquad\quad\|\\
\text{Y}\qquad\text{X}
\end{array}
$$

wherein:

E is carbon (C) or nitrogen (N);

W is hydrogen or a spacer group;

Y is hydrogen or a spacer group (when E is carbon], or a lone pair of electrons (when E is nitrogen];

S1 is a bond or a first spacer group;

S2 is a bond or a second spacer group;

S3 is a bond or a third spacer group;

X is oxygen (O) or sulfur (S);

B is a base segment comprising a nucleotidic base or an analog thereof; and

N is nitrogen.

In free NuAA monomers (i.e. before incorporation into a polymer), the N-terminus (i.e. the N with a free bond in the formula) is in the form of an amine or protected amine (e.g. a carbamate); and the C-terminus (i.e. the C with a free bond in the formula) is in the form of a carboxylic acid or, e.g., an ester.

Nucleotidic bases include the usual bases found in nucleic acids (i.e., the purine bases of adenine and guanine, and the pyrimidine bases of cytosine, thymine and uracil) and analogs thereof. A number of analogs of nucleotidic bases are well known in the art; many of which have been tested as chemotherapeutic agents. Some of these are described herein; see also, e.g., Beilstein's Handbuch der Organischen Chemie (Springer Verlag, Berlin), and Chemical Abstracts, which provide references to publications describing the properties and preparation of such compounds, which publications are incorporated herein by reference.

Thus, a large variety of analogs have been described that exhibit properties that may be advantageous in particular targeting schemes. For example, in some cases, it may be desirable to incorporate a base that binds non-specifically at a particular position. The base present in inosine is an example of such a non-specific analog. This can be used to incorporate degeneracy into the PENAM at particular positions which might be particularly useful, for example, in targeting a closely related family of target nucleic acids that are homologous except for one or a few positions in the base sequence. Inosine can pair with all four natural bases although the strength of binding varies: dC > dA > dG/T. See, e.g., [43].

Other types of modified bases that may be of particular interest are those which enhance binding affinity. For example, diaminopurine can form three hydrogen bonds with thymine, whereas adenine and thymine form only two [44]. Similarly, pyridopyrimidine bases can be used in place of cytosine to provide stronger base pairing with guanine [45].

Bases can also incorporate any of a variety of "target modifying groups". By way of illustration, base analogs can function as cross-linking moieties. For example, 6-bromo-5,5,-dimethoxyhexanohydrazide can be introduced into the C^4 position of cytidine to alkylate and thereby cross link guanosine [46]. N^4,N^4-Ethano-5-methyl-cytosine can be used to similar effect [47, 48].

Another example of base analogs that can be incorporated into a PENAM are fluorescing analogs such as the base of pyridopyrimidinedeoxynucleosides [45]. Such bases can thus be used as labeling groups.

A wide range of purine and pyrimidine analogs exhibiting various properties is known in the art [see, e.g., references 49-62]. An exemplary but not exhaustive list of such analogs includes: 1-methyladenine,

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1-methylguanine, 1-methylinosine, 1-methylpseudouracil, 2-methylthio-N^6-isopentenyladenine, 2-thiocytosine, 2-methyladenine, 2-methylguanine, 2-thiouracil, 2,2-dimethylguanine, 2,6-diaminopurine-3-methylcytosine,

5   3-(3-amino-3-N-2-carboxypropyl)-uracil-4-acetylcytosine, 4-thiouracil, 5-fluorouracil, 5-iodouracil, 5-bromouracil, 5-methyluracil, 5-methyl-2-thiouracil, 5-methoxyaminomethyl-2-thiouracil, 5-chlorouracil, 5-carboxymethylaminomethyl-2-thiouracil,

10   5-methylaminomethyluracil, 5-carboxyhydroxylmethyluracil, 5-carboxymethylaminomethyluracil, 5-methoxyuracil, 5-methylcytosine, 7-methylguanine, 7-deazaguanine, 7-deazaadenine, β-D-mannosylqueosine, β-D-galactosylqueosine, dihydrouracil, hypoxanthine,

15   inosine, N-uracil-5-oxyacetic acid methylester, N^6-methyladenine, N^6-isopentenyladenine, pseudouracil, queosine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, and xanthine.

     Particular spacer groups, e.g., at S1, S2, S3, W

20   and/or Y in the formula, are as described above. The incorporation of spacer groups at S1 and S2 will affect the relative distance between adjacent bases on NuAA monomers. Preferably, the distance between adjacent bases is at least about 4 carbon-carbon bond lengths,

25   more preferably at least about 5, still more preferably about 6. This spacing can be achieved by including a corresponding number of atoms between the E position of one NuAA monomer and the E position of the next NuAA monomer. Thus, to incorporate about 6 bond lengths, for

30   example, one can include 5 atoms along that length of the backbone. The desired "spacer" atoms can be incorporated within NuAA monomers (for example, by using monomers with a propylene moiety at S2 or S1, or e.g. a methylene at S1 and an ethylene at S2). Most preferably, however, S1 is

35   a bond since that will facilitate the synthesis of NuAA monomers from naturally occurring amino acids and their derivatives. As described below there are a number of

20

commercially available amino acids that can be used as
precursors for the synthesis of NuAA monomers having a
variety of spacer groups at S2. As described in Example
2, molecular modelling can be used to assess the likely
5   impact of, for example, lengthening the spacer group at
S2. Preferably, S2 is a methylene group, more preferably
an ethylene group, still more preferably a propylene
group.

Alternatively, the spacer atoms can be provided by
10 monomers located between NuAA monomers. Since aminoacyl
monomers derived from natural amino acids will provide a
three-atom backbone, these can be conveniently used
between adjacent NuAA monomers. Such spacer AA monomers
can also be used to provide D- or L-chiral centers.
15 Similarly, aza-AA monomers can be used to provide quasi-
chiral centers between adjacent NuAA monomers. Thus,
individual PENAMs can include one or more types of
monomers and/or chiral centers.

The spacer group at S3 is used to affect the
20 distance between the nucleic base and the peptidic
backbone. Molecular modelling has been used to show
that, in order to mimic the homomorphism of native
nucleic acids, the nucleic base is preferably substituted
on the side chain at least two carbon units distance from
25 the peptidic backbone (e.g., γ-substituted), and is
preferably not more than four carbon units away from the
backbone. Such NuAA monomers can be easily synthesized
from commercially available starting materials, for
example, natural alpha-amino acids, as illustrated below.
30 The atom at E will generally be a carbon, in which
case E can be a chiral center analogous to the alpha
carbon of a natural amino acid (other than glycine).
When E is a chiral center, then the NuAA monomers can be
L- or D-NuAA monomers depending on whether the
35 configuration of groups around the chiral center is
analogous to that found in an L- or D-amino acid (where
the base segment is in the position normally occupied by

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an amino acid "R" group and the carbonyl and amide groups are in the directions shown in the formula). As described herein, the PENAMs of the present invention contain D-chiral and/or quasi-chiral centers.

5   If E is carbon, then W and Y can be hydrogen or a spacer group as defined above. Most preferably, W and Y are hydrogen atoms.

When the atom at E is replaced isosterically with trivalent nitrogen in place of carbon, the resulting NuAA monomer (termed an "aza-NuAA monomer") can adopt 10   configurations between D- and L- amino acids. Thus, there will generally be a lone pair of electrons at position Y; and, since the electrons are not fixed, the resulting center around E can adopt a variety of spatial configurations and is referred to as a quasi-chiral 15   center.

NuAA monomers can be conveniently synthesized from generally available starting materials such as natural amino acids, unnatural amino acids and the derivatives thereof, see, e.g. [63-65]. As will be appreciated, a 20   variety of possible synthetic routes can be employed. "Retrosynthesis" techniques can be used to identify particularly convenient pathways; some of which are illustrated below.

25   As described below, the design of such mimics can be tested by well-known molecular modelling techniques in order to assess the likely impact of a particular group on binding of the targeting bases of the side chain to their nucleic acid target.

30

2.   Assembly of Particular PENAMs from NuAA Monomers

The PENAMs of the present invention contain strings of NuAA monomers (although they may also contain other groups, as described herein). The sequence of bases in 35   the PENAM can be made to complement any target nucleic acid sequence by simply incorporating the corresponding NuAA monomers into the PENAM. Thus, where a gene or

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other nucleic acid target has already been at least partially sequenced, then PENAMs can be designed to interact with such a target nucleic acid according to the standard "rules" of Watson-Crick base-pairing (i.e., a single-stranded nucleic acid comprising a nucleotide sequence CCGGTTAA could be targeted by a PENAM comprising a base sequence GGCCAATT; note that longer sequences are generally used, as described below).

Several basic considerations guide selection of a suitable number of bases to include. Two particularly important consequences of increasing the length of the complementary region are an increase in specificity and an increase in affinity. The increased specificity derives from the fact that the general probability of occurrence of a particular target sequence will be $(1/4)^n$, where n is the number of bases in the target sequence. Thus, the probability (P) that a particular sequence of n bases will occur in a nucleic acid containing a total of N bases can be estimated from the following formula:

$$P = N / 4^n$$

For example, the probability that a 10 base sequence will occur in a nucleic acid of 30,000 nucleotides (30 kb) would be estimated to be $3 \times 10^4 / 4^{10}$ or about 0.03; and the probability of a 15 base sequence occurring would be approximately 0.0000279. Thus, for targeting sequences in a viral genome, most of which have less than 300 kb of unique-sequence nucleic acid, a sequence of less than 15 bases, and perhaps as few as 9 or so, may provide more than sufficient specificity of targeting. In more complex genomes, it is preferable to have a longer sequence. Thus, a 17 base sequence would be expected to occur once in the entire human genome [8].

It is also the case that overall binding affinity is improved by providing more bases for hydrogen bonding. This explains the correlation observed in antisense ODNs between chain length and antisense activity. For duplexes in the range of 10 to 20 base pairs (bp), the

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melting temperature increases by roughly three degrees
Celsius for every additional base pair formed by two
hydrogen bonds (e.g., A:T) and by roughly six degrees
Celsius for every additional base pair formed by three
5   hydrogen bonds (e.g., G:C). Thus, a sequence number
selected on the basis of specificity [vis-a-vis the
complexity of nucleic acid in the targeted genome(s)] may
be extended to influence the affinity of binding.
However, the significance of the correlation between
10   length and affinity is greatly reduced once the number of
bases exceeds about thirty [8]. Furthermore, longer
sequences may be more difficult to synthesize and may
penetrate cellular membranes less efficiently. Of
course, a preferred approach will be to identify an
15   appropriate range, typically 10 to 25 bases, and then to
compare a set of oligomers of differing lengths within
that range to determine an optimum length. Synthesizing
a   range   of   PENAMs   of   differing   lengths   and/or
specificities can be easily accomplished using the
20   present invention since the PENAMs are designed to be
prepared   using   modular   synthesis   on   a   peptide
synthesizer.

The particular bases incorporated within the PENAM
will of course depend on the desired nucleic acid target.
25   As is well known to those of skill in the arts of DNA
probes and antisense technology, the overall binding of
the targeting molecule to its target depends, inter alia,
on the length of the complementary sequences and the
number of mismatches within those sequences. Preferably,
30   the PENAMs of the present invention will be at least
"partially complementary" (60% homologous) to a stretch
of bases in the target nucleic acid. More preferably,
the PENAM will be "substantially complementary" (at least
about 80% homologous); most preferably, the PENAM will be
35   "completely   complementary"   (100%   homologous).   As
discussed above, however, it is quite feasible to
incorporate special base analogs (such as inosine) to

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effectively broaden the binding capability for one or more positions in the monomer (if, for example, it represents a position that is unidentified, or is likely to vary in a given population of target sequences). It
5   is also feasible to incorporate other analogs to further enhance binding; see the illustrations above regarding the use of modified bases with enhanced affinity, and the use of cross-linking moieties.

PENAMs can also target double-stranded target
10 nucleic acids via triple helix formation, as discussed below.

It is also possible to combine the PENAM technology of the present invention with the use of standard oligo(deoxy)nucleotides (i.e., "ODNs"). Thus, for
15 example, a PENAM might include a sequence of "natural" nucleotides. However, since the predominant activity of exonucleases appears to be 3' to 5' it would be preferable to have at least the 3' end of such an ODN linked to another portion of the PENAM.

20

Homomorphism

The PENAMs of the present invention have a peptidic backbone that incorporates unusual chiral centers
25 (including D-chiral centers and/or quasi-chiral centers) that can be used to orient the nucleic side chains in such a way that the nucleotidic bases are spatially homomorphic to bases in targeted nucleic acids. The ability to enhance binding by spatial homomorphism is
30 especially significant given that hydrogen bonding interactions between biomolecules typically depend on an aggregation of many relatively weak bonds.

For optimal binding, it is believed that the PENAM should have a conformation in which the nucleotidic bases
35 are stacked approximately at the 3.5 Angstrom interval and are oriented away from the backbone in such a manner that the hydrogen bond donors and acceptors of the

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nucleotidic bases can directly "read" the complementary
bases on the target nucleic acids by specific hydrogen
bonding interactions. That is, the PENAM and the target
should be substantially homomorphic. In order to achieve
5   such homomorphism, the PENAMs of the present invention
comprise strings of NuAA monomers (and may comprise other
intervening monomers) in which the NuAA monomers (or the
intervening monomers, or both) can incorporate unusual
chiral centers, including D-chiral centers and/or quasi-
10   chiral centers.

The various monomers and chiral centers can be
incorporated into PENAMs in a variety of different
configurations.   Thus, PENAMs can incorporate, for
example, mixtures of L- and D-chiral centers; which can
15   be in unequal proportions or in roughly equal
proportions. PENAMs with roughly equal proportions of L-
and D-chiral centers can have these centers dispersed
(e.g. wherein at least about 85% or L-chiral centers are
flanked by D-chiral centers). Higher proportions of D-
20   chiral centers can also be used (e.g. wherein at least
about 80% of the centers are D-chiral centers). Quasi-
chiral centers can be incorporated in addition to, or in
place of, D- and/or L-chiral centers; and these allow the
resulting PENAM to adopt a variety of spatial
25   conformations at the quasi-chiral centers.   Molecular
modelling with energy minimization of PENAM strands in
association with a target DNA strand, as illustrated
below, can be used to predict the preferred positioning
of the particular chiral centers and/or quasi-chiral
30   centers for a particular PENAM structure.

NuAA monomers can be joined directly to each other
or they can be separated by intervening monomers of other
types.   Preferably, such other monomers (or strings
thereof) are amino acids and/or aza-amino acids, or their
35   derivatives, since all of these monomers can then be
conveniently   polymerized   via   peptide   synthesis
techniques.   Of course, it will also be possible to

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separately prepare larger fragments, such as polypeptide fragments, and then incorporate such fragments into a PENAM.

As discussed herein, the peptide backbone of the
5   PENAMs of the present invention serves as a scaffold to orient the side chains (-S3-B in the formulae) in such a way that the nucleic bases substituted in the side chain can "read" the complementary nucleic bases sequence on the target nucleic acid. From the rational drug design
10 point of view, the peptide backbone scaffold can be c h e m i c a l l y   m o d i f i e d   t o   a l t e r   i t s hydrophobicity/hydrophilicity, or its electronic and/or stereochemical properties [66-68]. An exemplary but not exhaustive list of such chemical modifications includes:
15 1) modifications of the amide nitrogen; 2) modifications of the alpha-carbon (where E is carbon); 3) modifications of the amide carbonyl; and 4) modifications of the amide bond. Decreasing the hydrophilicity of the backbone can be expected to improve uptake of the molecules.
20 Decreasing the electronegativity of the backbone can also be expected to enhance binding to a complementary nucleic acid (which is itself electronegative). In these regards, the PENAMs of the present invention (in which the polyphosphate-sugar backbone has been entirely
25 replaced) are already substantially improved relative to standard ODNs. However, additional modifications affecting hydrophilicity/electronegativity can also be incorporated. As will be apparent to those of skill in the art, the incorporation of cationic or anionic amino
30 acids as spacer AA monomers would render the PENAM cationic or anionic, respectively. Thus, "basic" amino acids such as lysine or arginine can be conveniently used to render the PENAM cationic in nature, and "acidic" amino acids such as aspartate or glutamate can be
35 conveniently used to render the PENAM anionic in nature. Making the PENAM cationic would of course be expected to electrostatically enhance the interaction between the

PENAM (cationic) and the phosphate backbone of DNA/RNA (anionic). Modifications in the backbone (especially at S2 and/or S3) can be used to favor particular conformations (e.g. helix vs. beta-sheet) and to thereby improve base stacking.

Molecular modelling techniques can be used to assess the likely impact of a particular group (such as a larger spacer group) on binding of the targeting bases of the side chain to their nucleic acid target. A number of suitable molecular modelling techniques are known in the art [69-75], and computer software incorporating such techniques is also available, as described below.

Molecular modelling, often synonymous with computer-aided molecular design, is a general methodology which comprises several computer-based methods for drug design [76, 77]. Using these methodologies, one can readily assess the impact of introducing various modifications into targeting molecules such as the PENAMs of the present invention. A number of publicly available modelling packages, including BIOGRAPH-II*, CHEMLAB-II*, CHEM-X*, HYPERCHEM*, MACRO MODEL*, SYBYL*, and others, can offer some or all of the following advantages that can be used to design and check particular PENAMs of the present invention. Some of the useful features of these systems are outlined below.

Model building and computer graphics programs allow users to "build" a molecule from scratch or import it from 3D structure data bases and also allow manipulations of the displayed model, ranging in size from small molecules to macromolecules in 3D. They also enable the user to dock more than one ligand into the putative active site of the macromolecule and to manipulate the display simultaneously or individually. Publicly available programs of this type include, for example: ALCHEMY*, from Tripos associates; MIDAS PLUS*, from The University of California, San Francisco; FRODO*, from Rice University, Houston; HYDRA* from Polygen; MANOSK* from

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University of Paris; MOLECULAR GRAPHICS° from ACS software; and MOGLI° from Evans and Sutherland. Molecular model building programs like CONCORD° and COBRA° can also be used to generate low energy 3D structures from 2D on
5   work stations [78].

Molecular mechanics and dynamic simulation programs allow model refinement by optimizing the rough coordinates of the model, utilizing either quantum mechanics or molecular mechanics calculations. Molecular
10   mechanics can optimize the 3D structure of a molecule by moving to the nearest local minimum energy structure. Even though both quantum mechanics and molecular mechanics have their basic differences in calculating the potential energy function of an isolated molecule or a
15   biological system, they complement each other and can be used to optimize the interaction energy and the binding conformation between the ligand and its target macromolecule [79, 80]. Molecular dynamics simulations can be used to follow the solvation and conformational
20   changes involved in the interactions between the ligand and its receptor on initial binding, and also any conformational or covalent rearrangements which may occur on subsequent binding. Molecular dynamics can be used to assess entropies, enthalpies and other thermodynamic
25   quantities for various molecular configurations [81]. Accordingly, these calculations can be used to predict how variations in the basic structure of a PENAM will affect the equilibrium constant for binding to its target.   Publicly available programs of this type
30   include, for example: MM2° and MOPAC°, from Quantum Chemistry Program Exchange; and MMP2°, from Molecular Design Ltd.   In addition, AMBER° and GROMOS°, from The University of California, San Francisco, are two programs available for molecular mechanics and dynamics
35   simulations [80].

Docking, scoring and screening algorithms can also be used as computer screening procedures to test lead

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candidates for binding to putative ligands. The docking
criteria typically involve either geometric orientation
or steric match and evaluating the goodness of fit of the
"ligand" to the active site. The scoring algorithms use
5   steric complementarity, full intermolecular force field
and other molecular properties as the scoring functions.
Publicly available programs of this type include, for
example: DOCK*, from The University of California, San
Francisco [82-84]; GEOM*, from the Cambridge Structural
10   Data Center; ALADDIN*, from Daylight chemical Information
Systems, and MACCS-3D* from Molecular Design Ltd. [78].
       Quantitative structure-activity relationships (QSAR)
and statistical programs can be used to correlate the
biological potency of a series of analogs with the
15   relative values of physical properties such as partition
coefficients (log P), pK_a values, and electronic or steric
properties and the size of the substituent. In addition
to QSAR, the comparative molecular field analysis
(CoMFA), which is a combination of receptor mapping,
20   potential energy calculations and QSAR, uses the relative
potency of molecules and their superimposed bioactive
conformations to calculate steric, electrostatic and
hydrogen-bonding interaction energies for each molecule
[85, 86]. Statistical analysis of these energy values
25   with respect to their biological potency or affinity can
be used to predict the affinity of the proposed molecule
for a given target. Publicly available programs of this
type include, for example: MEDCHEM*, a modelling and
information management program developed by Corwin Hansch
30   at Pomona college [87], which includes algorithms to
calculate log P values; and ADAPT*, another complete
program, developed by Jurs et al. [88] and marketed by
Molecular Design Ltd., which performs all the operations,
i.e., vector representation of the molecules, statistical
35   analysis by various methods, data input and management.
       As an illustration of these techniques, Example 1
describes the use of molecular modelling to analyze the

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stereochemical impact of changes to the chirality of the peptidic backbone in the PENAMs of the present invention. In particular, a model of the PENAMs of the present invention was prepared in which the peptide backbone

5 comprises alternating chiral centers, and this was compared to a model in which the backbone was assembled from "natural" L-amino acid-like monomers. As described in the example, the modelling data indicate that the ability to incorporate centers of varying chirality

10 substantially improves the homomorphism or "fit" of the PENAM to its target nucleic acid. As described herein, centers of varying chirality including D-chiral, L-chiral and quasi-chiral centers can also be introduced into spacer monomers such as amino acids, incorporated between

15 adjacent NuAA monomers. Of course, the inclusion of small achiral amino acids such as glycine as spacers would be expected to have the least effect on the overall configuration of the PENAM; whereas the inclusion of L- and/or D-amino acids can be used to alter the

20 conformation of the PENAM.

It is known that DNA and RNA can exist in several secondary structures or isoforms such as right-handed A- and B-forms, as well as the left-handed Z-form, and it is believed that these isoforms could be essential for

25 specific biological functions [89]. These polymorphisms in the double helix arise from differences in base stacking, backbone geometry, and the sugar conformation of the particular form. Each of these forms differ distinctly from each other with respect to helical

30 hardness, as well as the shape and size of the helical groove. These distinct features have been effectively exploited to design conformation-specific metal complexes by Barton et al. [90]. Like synthetic ODNs, the PENAMs of the present invention are expected to be capable of

35 binding to each of the various isoforms of the nucleic acids [91-93]. The molecular modelling techniques described above can be effectively used as a selection

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tool to assess the "homomorphic fit" of a particular
PENAM to a generated molecular model of a specific
nucleic acid isoform.

5 Assembly of PENAMs from NuAA Monomers

Since the NuAA monomers can be joined via peptide
bonds, the PENAMs of the present invention can be
conveniently assembled using well-known peptide synthetic
techniques. These building block NuAA monomers thus
10 allow for the rapid production of a variety of
"antisense" oligomers, exhibiting differing sequences for
targeting complementary nucleic acids, via an automated
peptide synthesizer.

Solid phase peptide synthesis (SPPS) is a convenient
15 and powerful methodology for the synthesis of a wide
variety of oligopeptides in large quantity and high
purity [94]. The methodology involves three basic steps:
(i) peptide chain assembly; (ii) peptide cleavage, side
chain group(s) deprotection and isolation from polymeric
20 support; and (iii) purification and characterization of
the oligopeptide.

The two most common synthesis strategies for SPPS
are based on the deprotection chemistry of either t-Boc
or Fmoc groups, used for the protection of the amino (NH$_2$)
25 group of the amino acid. The choice of t-Boc or Fmoc
depends upon the protection and deprotection chemistry of
the functional groups of each monomer.

The first step in chain assembly is to attach the
α-carboxyl group of the first t-Boc- or Fmoc-protected
30 amino acid or aminoacyl monomer through a linker to a
solid polymeric support. The next step is deprotection
or removal of the t-Boc or Fmoc protective group of the
first amino acid. The t-Boc protecting group is removed
by an acid, usually trifluoroacetic acid (TFA), whereas
35 Fmoc group is deprotected by base, usually piperidine.
After deprotection, the next amino acid is coupled to the
deprotected amino (NH2) terminus through its α-carboxyl

(-COOH) group to form a peptide bond. Through successive
cycles of deprotection and coupling, the growing peptide
is assembled from the C-terminal towards the N-terminal
to a desired chain length. Once the desired chain length
5   is achieved, the oligopeptide is cleaved from the
polymeric support either by a strong or weak acid. Also,
depending upon the type of the resin linkage on the
polymeric support, the cleaved oligopeptide has free
carboxyl or carboxyamide at the C-terminus.

10 As described herein, the PENAMs of the present
invention are unlikely to be degraded by either cellular
nucleases or peptidases because of their unique structure
which no longer resembles either nucleic acids or natural
peptides. In addition, the uncharged backbone of PENAMs
15 facilitates transport across the cell membrane. Uptake
and transport across cellular membranes can be further
enhanced by conjugating the PENAMs with carrier molecules
at the N-terminus, at the C-terminus, or at an internal
position in the PENAM. Carrier molecules include small
20 reactive chemical groups (e.g., fluorescent dyes,
intercalators, cross linking agents, alkylating agents,
chain cleaving agents, biotin, digoxigenin, cholesterol,
etc.) or stretches of other peptide-based compounds,
including natural, modified or synthetic oligopeptides,
25 or proteins (e.g., peroxidases, IgG, alkaline
phosphatases and nucleases). They can be readily joined
to the PENAMs via peptide bonds either during the
synthesis or at the end of the synthesis on the peptide
synthesizer [9]. Various strategies known in the art can
30 be used to facilitate the uptake and selective
subcellular localization of the PENAMs [95-109]. These
strategies include:
1) increasing hydrophobicity by chemical modifications
(for example, insertion of small chemical groups such as
35 methylene, ethylene, propylene at S1 or, more preferably
at S2 in a NuAA monomer) or by appending lipophilic

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groups such as cholesteryl and its derivatives and/or other long chain fatty triglyceryl residues;

2)   physical or chemical association with polycations (for example, biotinylated molecules complexed to avidin can also exhibit increased cellular uptake, most probably due to the increased binding to the negatively charged cell surface (polycations would also enhance the binding of the PENAM to negatively-charged DNA);

3)   conjugation to ligands recognizing membrane proteins and receptors (for example conjugation to mannose-6-phosphate substituted serum albumin or mannosylated streptavidin); of course, conjugation to ligands that associate with cell-specific receptors (e.g. conjugation to a cytokine that binds only to cells bearing a cognate cytokine receptor) can be used to specifically "target" the PENAM to a desired sub-population of cells;

4)   delivery through nanoparticles made up of biodegradable polymers, such as polycyanoacrylate;

5)   poly(L-Lysine) mediated delivery; and

6)   association with lipoprotein or liposomes.

Tandem Targeting

A modification of the approach that may be especially effective is the use of "tandem targeting" in which more than one different oligomer is employed as described by Maher and Dolnick, and Goodchild et al. [110, 111]. In particular, the use of two or more distinct targeting sequences (complementary to adjacent regions of the target) may result in synergistic effects on the modulation of the target. Preferably, the gap between the adjacent regions of complementarity will be less than about 15 bases, still more preferably the gap will be less than about 2 bases, and most preferably there will be no gap.

34

Triple Helix Formation

Another way of targeting double-stranded DNA is via triple helix formation. There are several different motifs which are known to facilitate triple helix
5 formation. In the "CT" motif, G-C basepairs are recognized by C residues and A-T basepairs are recognized by T residues - resulting in T-A-T and C-G-C* triplets across the three strands of the triple helical structure. This system is effective where one strand of the
10 double-stranded target sequence contains only, or mostly, purine bases. An exception which may allow for a number of pyrimidine residues in the target sequence has been described by Griffin et al. [112]. The pyrimidine-rich targeting oligomer associates with the duplex such that
15 the polarity of the oligomer is parallel to that of the strand containing the purine-rich target sequence. In situations in which a shorter purine-rich stretch is adjacent to a pyrimidine stretch, the oligomers may be designed to possess corresponding regions of inverted
20 polarity such that the oligomer binds to the purine-rich stretch of one strand and then binds to the adjacent purine-rich stretch of the opposite strand. The recognition rules of the CT motif are further described by, for example, Maher et al. [113]; and Moser et al.
25 [114]. One problem that may affect the use of the CT system is the ionization state of the targeting C residue at physiological pH. In order to facilitate the appropriate hydrogen bonding, the amino group at position 3 of the C must be protonated. This presents no problem
30 when the pH is low; however, at neutral pH, most of the pyrimidines are unprotonated. If the CT system is to be used at physiological pH, it may be preferable to employ 5-methylcytosine or 5-bromouracil in place of cytosine; as described for example by Povsic et al. [115]; and Lee
35 et al. [116]. It may also be especially useful to enhance stability of the triple helix by employing a

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PENAM which comprises a group which will cross-link or intercalate into the target double helix.

In triple helix formation via the "GT" motif, guanine residues recognize G-C pairs, and thymine or
5   adenine residues recognize A-T pairs, as described for example by Cooney et al. [117]. In the GT motif, the targeting oligomer is in an anti-parallel orientation vis-a-vis the target sequence. Thus, via one or another sort of triple helix formation, a PENAM can be used to
10 target a complementary sequence on one strand of a double-stranded target [118-137]. The efficiency of Hoogsteen binding can be further enhanced by a number of well-known techniques. For example, as discussed above, 5-methylcytosine or 5-bromouracil in the targeting
15 sequence can be used to enhance binding to complementary G residues in the target sequence. Other approaches to alter the biologic effects of triple helix formation include the introduction of modifying groups onto the targeting nucleic acid or PENAM, such as intercalating or
20 crosslinking groups. For example, an acridine derivative can be introduced onto an end of a targeting sequence to enhance triple helix stability. These and other modifications are further described in Matteucci and Bischofberger and citations therein [2].

25

Variations of Basic PENAM to Modify Target

As discussed herein, a variety of functional groups can be introduced into the basic PENAM structure in order to further affect the interaction between the PENAM and
30 its target nucleic acid, and/or to affect the target nucleic acid itself. These reactive groups can be appended either at the N-terminus or at the C-terminus or at an intervening position. Examples of such reactive groups, discussed herein and in the art include labeling
35 groups, intercalating groups, cleaving groups and other groups that reconform or bind to nucleic acids or modify the target nucleic acid [3, 9].

36

One type of modifying group that can be introduced into a PENAM is a nucleic acid intercalating group. A number of such intercalating groups are known in the art, many of which are acridine derivatives [138-148].

5   Another type of modifying group is a cross linking group. Cross linking can be used to stabilize the interaction between a PENAM and its target, which may be especially useful in achieving and stabilizing triple helix formation. Various approaches to the stabilization

10   of triple helix formation include photochemical crosslinking as described by Le Doan et al. [149] and Praseuth et al. [150], and alkylation of the N7 of specific guanines in the target duplex as described by Vlassov [151] and Fedorova et al. [152].

15   Crosslinking can also be used to covalently link a new molecular structure, attached to the PENAM, to a particular location within a nucleic acid target. Thus, for example, a label attached to a PENAM could be linked to the particular location targeted by the PENAM. Such

20   labels could be photo-induced cross-linking agents, for example psoralen, coumarin, ellipticine and their derivatives [153-160].

Other labels might not involve a cross-linking group. A number of such labeling groups are known in the

25   art [161-166]. The ability to label particular sequences in a nucleic acid will also be useful in efforts to map and sequence various genomes.

Another type of modifying group that can be introduced into the PENAMs of the present invention are

30   nucleic acid alkylating agents. A number of such alkylating groups are known in the art. For example these include N-mustards as reactive alkylating compounds [167-177], porphyrins [161, 178], psoralens as photochemical activatable agents [158, 179] and quinones

35   as inducible alkylating agents [180].

Another type of modifying group that can be introduced into the PENAMs of the present invention are

nucleic acid cleaving groups. There are a number of
cleaving groups that can be used to allow a PENAM to act
as an artificial sequence-specific nuclease, which have
been described in the art [125, 181-192]. The following
5 approaches are intended as an illustrative, not an
exhaustive, list of cleaving groups. In one approach,
iron(III) EDTA is used as a cleaving group which
generates free radicals under appropriate redox
conditions as described by Moser et al. [114]. Other
10 redox-activated transition metal cleaving groups include
complexes of o-phenanthroline-Cu(I) introduced by
Francois et al. [193, 194] and porphyrins-Fe(II) used by
Le Doan et al. [195]. These systems may be more useful
in vitro, where redox activation is more readily
15 controlled. Another alternative is photochemical
cleavage as described by Perrouault et al. [154]. Still
another approach is to incorporate as a cleaving group a
relatively non-specific nuclease such as DNase I or
staphylococcal nuclease and effectively convert it into
20 a specific endonuclease by conjugation to the sequence-
specific PENAM, analogous to the work reported by Schultz
et al. involving ODNs [196-198]. Yet another possible
approach to cleaving target nucleic acids is to
incorporate a ribozyme into the PENAM [199-201].
25   Depending on the particular modifying groups
selected, these groups can be incorporated into the PENAM
at virtually any position, including positions within and
outside of the NuAA monomers. However, there are a
number of general considerations that should guide
30 selection of a particular group and location. The most
significant consideration is that the group should not be
introduced into a position that is likely to prevent
sufficient hydrogen bonding between the bases of the
PENAM and those of the target nucleic acid. Thus, while
35 small modifying groups can be accommodated within the
NuAA monomers engaging in hydrogen bonding to the target,
larger groups may be better accommodated outside of the

hydrogen bonding monomers. Even large modifying groups such as nuclease enzymes can be attached to such terminal regions of the PENAMs [8]. In some cases, the nature of the interaction between the modifying group will dictate

5 favorable positions in the PENAM. Moreover, molecular modelling can be used to anticipate favorable positions for the incorporation of such groups.

The binding affinity of candidate PENAMs can be readily tested using any of a variety of well-known

10 techniques. These include, for example, standard melting temperature (T_m) measurements [202-204]; as well as chemical, photochemical, physical and/or enzymatic probing [205-213]. Binding can also be effectively probed using "footprinting" techniques. Footprinting was

15 primarily introduced by Galas and Schmitz [214] to detect contacts between DNA and DNA binding proteins. The basic idea behind this methodology is to allow a protein to bind to radioactively-labelled DNA/RNA containing the sequence that is recognized by the protein. After the

20 binding event, the protein-DNA complex is subjected to nuclease digestion, usually using DNAse I. The regions of the DNA molecule covered by the protein are protected from the digestion while the rest of the DNA backbone is digested. The products of this cleavage produce blank

25 regions or "footprints" at the sequence where the protein was bound to the DNA sequence (as visualized on autoradiographs of electrophoretic gels such as those used for DNA sequencing). Thus, by comparision with a DNA sequence marker, one can identify the sequence

30 specificity of a particular DNA binding moiety. Various chemical reagents have been developed to enhance fingerprinting analyses. Among them, reagent systems consisting of [Fe(EDTA)$^{-2}$], hydrogen peroxide and sodium ascorbate [215-217] and gamma rays [218] are noteworthy.

35 Numerous examples of this technique to study the sequence-specificity of nucleic acids by oligonucleotides are known in the art [128, 148, 219-221].

39

UTILITY AND ADMINISTRATION

Diagnostic/Analytical/Preparative Applications:

The peptide-based nucleic acid mimics (PENAMs) of
5   the present invention are substantially homomorphic with
nucleic acids. This homomorphism facilitates hydrogen
bonding and thus hybridization with target nucleic acid
sequences.

Diagnostic, analytical and/or preparative uses of
10 PENAMs include the use of PENAMs as "reporter" molecules;
use of PENAMs for the capture of complementary DNA/RNA
sequences. For example, the PENAMs can be used as
hybridization probes to identify target sequences in a
particular DNA or RNA of interest [222-233].
15

Use as a Reporter

Generally, for diagnostic applications, the PENAMs
will comprise a labeling or "reporter" group (as
described herein) by which the interaction with the
20 proper target sequence can be detected [234-242]. A
typical example of such an application would involve a
pool of single-stranded polynucleotides (the sample or
"analyte" which might contain a target sequence such as
a viral nucleic acid). In a typical probe approach, this
25 pool of polynucleotides will be bound to a solid support.
A labeled PENAM is then introduced to the polynucleotides
under hybridizing conditions and allowed to anneal.
After washing away non-hybridized PENAM, the sample bound
to the solid support is analyzed for the presence of the
30 labeling group. Such assays can be carried out according
to standard hybridization procedures suitable for the
degree of complementarity expected. That is, high
stringency hybridization can be used for high levels of
complementarity (e.g. greater than 90%), lower levels of
35 stringency for lower levels of hybridization, as is well-
known in the art. However, given the reduced charge
repulsion between PENAMs and nucleic acids, and also the

40

possibility of introducing intercalating or cross-linking groups into the PENAMs, it will be possible to design PENAM probes that can be subjected to even more stringent hybridization conditions (e.g., higher temperature, lower

5   salt) than the corresponding native complementary strand. The ability to use more stringent conditions will reduce the background "noise" caused by non-specific or mismatched binding and, as a result, will reduce the incidence of false-positive results. For a given PENAM

10 and target, the optimum hybridization conditions can be determined by testing the PENAM under varying conditions (particularly the concentration of denaturants, the temperature during hybridization and washing, and the concentration of salts during washing) with both positive

15 and negative controls (known amounts of target sequence decreasing to zero).

Use for Capture

In another type of diagnostic/analytical/preparative

20 application, the PENAMs can be linked to a solid support for capture of complementary (target) nucleic acids to the support [243-245]. In such systems, the PENAMs can also be synthesized directly onto the solid support. The presence of target nucleic acid bound to the solid

25 support via the PENAM can be detected, for example, by using a polycationic molecule which would bind to the negatively charged backbone of the target nucleic acid (but not the peptidic backbone of the PENAM). The polycationic molecule can comprise a labeling group to

30 produce a signal by which the bound complex can be detected. The PENAMs of the present invention can also be employed in the variety of sandwich hybridization assays which are known in the art. As discussed above, one advantage of using PENAMs is the ability to run the

35 assays under more stringent hybridization conditions. The use of PENAMs as capture probes can also be applied to preparative techniques in which the PENAM,

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typically bound to some support, is used to attract and thereby retrieve a complementary target nucleic acid using methods analogous to those described for the diagnostic assays above.

5

Therapeutic Applications:
Antisense and/or Antigene Modulation

The PENAMs of the present invention are also useful, as described herein, for modulating the activity of
10 nucleic acids in an antisense manner. The ability to modulate nucleic acid activity by antisense regulation is well known in the art as described [5, 8, 121, 246-250]. With respect to the control of gene expression, PENAMs can be used not only to inhibit expression but also to
15 activate it in vitro as well as in vivo. Indirect activation of gene expression can be accomplished, for example, by suppressing the biosynthesis of a natural repressor, as described for antisense ODNs by Inoue [251]. Direct activation of gene expression can be
20 accomplished, for example, by reducing termination of transcription as described for antisense ODNs by Winkler et al. [252]. There are several in vitro as well as in vivo test systems known in the art that have been routinely used [95-97, 101, 109, 253-265]. The efficacy
25 of PENAMs as antisense or antigene chemotherapeutics can be easily tested and compared using these test systems

The ultimate targets of antisense modulation include viral diseases, bacterial diseases, inborn errors of metabolism, malignant cell growth, and any other
30 conditions associated with the presence of a characteristic DNA or RNA or products thereof [7, 266-270].

Preparation/Administration
35 Depending on the nature and location of the target nucleic acid, there are a number of different techniques that can be used for promoting contact between a PENAM

and its target. For example, where the target nucleic acid is in solution in vitro, the PENAM can simply be added to the solution and the mixture incubated under standard conditions favoring nucleic acid hybridization.

5 Where the target is intracellular, the PENAM must be capable of passing through one or more cellular membrane systems in order to contact the target nucleic. In this regard, the PENAMs of the present invention are already substantially more effective than nucleic acid based

10 targeting molecules such as antisense oligodeoxynucleotides (ODNs). In particular, the passage of ODNs through cellular membranes is very inefficient because of their highly-charged backbone. In the case of PENAMs, the bases are attached to a peptidic backbone

15 which is far less charged at physiological pH. If desired, the permeability of PENAMs can be further enhanced by incorporation of additional modifications. A number of such modifications are known in the art and have been described in detail earlier [2, 4]. These

20 modifications include, for example, the attachment of hydrophobic chains to the targeting molecule [271, 272]. Another approach involves the attachment of polylysine [105], however, polylysine may not be applicable in all cell systems [4]. Another non-specific carrier which can

25 be used to improve uptake is cholesterol and its derivatives [102, 273, 274].

Another approach is to utilize receptor-mediated endocytosis to enhance and/or to direct cellular delivery [2, 4]. In this approach, cells with receptors for a

30 particular ligand can be targeted by linking the ligand to the PENAM. By way of example, linking biotin, folate, transferrin or interleukin-1-β (IL-1B) to a PENAM of interest, one can specifically and efficiently target cells expressing biotin, folate, transferrin or IL-1B

35 receptors. For situations in which the receptor status of a cell type is unknown, or where a receptor-ligand pair is known but the ligand cannot be suitably linked to

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the PENAM, it may also be possible to use more
generalized ligands such as lectins (sugar-binding
proteins) since most cells are believed to have
glycoproteins on their surface. Such a generalized
5   approach may also be useful where targeting a broad range
of cellular types is desired. Analogous approaches can
be used to target nucleic acids within a cellular
organelle. For example, linking a mitochondrial protein
to a PENAM can be used to target the PENAM to
10   mitochondria as has been described for incorporating
double-stranded DNA into mitochondria [4]. Of course,
such receptor-mediated delivery systems may utilize any
of a large number of other known cellular receptor
systems.

15   Still another approach is to deliver the PENAMs to
target cells using liposomes [98] or antibody targeted
"immunoliposomes" [2]. Linking of acridine or
benzophenanthridine to the PENAM can be used to improve
incorporation into neutral liposomes [4].

20   The PENAMs are thus useful in therapeutic,
diagnostic and research contexts. PENAMs can be
administered in the same manner as modified ODNs, which
manner of application is conventional in the art. In
therapeutic applications, the PENAMs are utilized in a
25   manner appropriate for antisense therapy in general. For
such therapy, the PENAMs can be formulated for a variety
of modes of administration, including systemic and
topical or localized administration. Techniques and
formulations may be found, for example, in Remington's
30   Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.
(latest edition). The PENAM active ingredient is
generally combined with a carrier such as a diluent or
excipient which may include, for example, fillers,
extenders, wetting agents, disintegrants, surface-active
35   agents, or lubricants, depending on the nature of the
mode of administration and the dosage forms. Typical
dosage forms include tablets, powders, granules,

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capsules, suppositories, liquid preparations such as suspensions, emulsions and solutions, and liquid preparations for injections, including liposome preparations. For systemic administration, injection is

5 preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the PENAMs of the invention are formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In

10 addition, the PENAMs may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included. Systemic administration can also be by transmucosal or transdermal means, or the compounds can be administered orally. For

15 transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, bile salts and fusidic acid derivatives for transmucosal administration. In

20 addition, detergents may be used to facilitate permeation. Transmucosal administration may be through use of nasal sprays, for example. For oral administration, the PENAMs are formulated into conventional oral administration forms such as tablets,

25 capsules and tonics. For topical administration, the PENAMs of the invention are formulated into ointments, salves, gels or creams, as is generally known in the art.

The PENAMs of the present invention will, by virtue of their unusual structural features, be resistant to the

30 degradative enzymes that are expected to be present in most biological systems. Thus, the PENAMs do not possess the phosphodiester backbone which is the standard target of the nucleases. Also, the peptidic backbone is unlike that of naturally-occurring peptides because of the

35 presence of both D- and L- residues. This basic resistance to degradative enzymes can be further enhanced, if desired, by the addition of protecting

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groups which interfere with the ability of a degradative
enzyme to modify the oligomer.

Examples

5   The following examples are intended to illustrate,
not to limit, the invention.

General procedures

       Unless otherwise noted, the following general
10 procedures were employed in conducting the experiments
subsequently described:
       All moisture-sensitive chemical reactions, requiring
inert atmosphere, were carried out under nitrogen or
argon in oven- or flame-dried glassware using septum
15 technique. All chemicals and solvents were of technical
or reagent grade and used as received unless otherwise
stated. Thin layer chromatography (tlc) was carried out
on pre-coated 0.25 mm layer thickness silica gel and/or
alumina plates, supplied by Analtech Co. Preparative
20 thin layer plates were coated with 1.5 mm layer thickness
silica gel GF-254 (60 mesh, ASTM). Flash column
chromatography was carried out using 70-230 mesh silica
gel, supplied by E. Merck. Melting points were taken on
an electrothermal apparatus and are uncorrected.
25 Infrared spectra were recorded on a Nicolet FT-IR
spectrophotometer with samples prepared as potassium
bromide pellets or as thin films on NaCl plates.
Ultraviolet spectra were recorded in quartz cuvettes on
Hewlett Packard UV/VIS spectrophotometer. NMR spectra
30 were recorded on GE 300 MHz or GE 500 MHz spectrometers
using deuterated solvents and tetramethylsilane as an
internal standard. Mass spectra were recorded at UCSF
Mass Spectrometry center. Optical rotations were
measured in a 1-dm quartz cell at 22-23 degrees Celsius
35 at the sodium D line on a Perkin-Elmer polarimeter (Model
241 MC). Solid Phase Peptide Synthesis was carried out
on an Applied Biosystems Peptide synthesizer (model

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431A), using Boc-strategy. Hydrogen fluoride setup was
used to cleave the peptides from the resin. The peptides
were purified either by HPLC using C18 reverse phase
Vydac® column or by FPLC using cation exchange Mono S°
5 column.

Example 1
Basic molecular comparison of PENAMs of the present invention with standard ODNs and with PENAMs that do not
10 contain centers of varying chirality

As shown in Figure 1, the chimeric PENAMs of the
present invention are structurally quite distinct from
standard oligonucleotides (ODNs). It is by virtue of
these differences that PENAMs avoid a number of technical
15 difficulties associated with the transport and stability
of ODNs. Functionally, however, the PENAMs are analogous
to ODNs in their ability to engage in sequence-specific
hydrogen bonding to complementary nucleic acids by virtue
of their nucleotidic sidechains being properly
20 "presented" by the peptidic backbone. Molecular
modelling has been used to analyze and confirm these
structural and conformational relationships.

Design and model building computations were
performed on a Silicon Graphics (Mountain View, CA) IRIS
25 work-station [275] using the SYBYL° molecular modelling
package (v.5.4/6.0), offered by Tripos Associates [276].
There are several ways to generate or build these models.
As an illustration, the following approach has been used
to build comparative models

30 Firstly, to illustrate the substantial structural
differences between the PENAMs of the present invention
and standard ODNs, molecular modelling was used to show
the steps required to "convert" DNA into a PENAM. The
SYBYL DNA sequence builder was used to generate the
35 complementary double stranded DNA decamer, $dT_{10} \bullet dA_{10}$, into
B-DNA conformation, based on the crystal structure,
solved by Arnott et al. [277]. The purine strand (dA_{10})

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of the generated DNA decamer, $dT_{10} \bullet dA_{10}$ was then used as a template to construct examples of the PENAMs: Version I (without varying chiral centers) and Version II (with alternating chiral centers).

5   First, the oxygen atoms (O4') of the ribose rings, along with the C4'--O4' and O4'--C1' bonds, were deleted. Then, all of the O3' oxygen atoms and C4' carbon atoms were substituted by carbonyl (C=O) groups and amide nitrogens (N), respectively. Next, the C5' carbons, the
10   O5' oxygens and the remaining phosphate backbone (⁻O-P=O) were deleted. Next, trans-amide bonds were made between the amide nitrogen (N4') of each monomer and the carbonyl (C=O3') of the next monomer. Appropriate hydrogen atoms were added on all of the modified atoms (C1', N4') to
15   satisfy their hybridization states. Having effectively transformed the sugar-phosphate backbone into a peptidic backbone, the side chains were then substituted with the nucleic bases (adenine in this illustration), to generate a chimeric peptide-based nucleic acid mimic.

20   In order to compare the homomorphism of PENAMs with and without varying chiral centers, two different versions of the previously described PENAM were constructed. In PENAM Version I (without varying chirality), all of the alpha carbons (C2) were assigned
25   the L-configuration as in the "natural" L-amino acids. PENAM Version I was then energy minimized as a single strand, in beta-sheet-like extended conformation, using force field parameters offered by SYBYL software package.

In PENAM Version II, all alpha carbons (C2) of odd
30   numbered residues were assigned the L-configuration as in "natural" amino acids, and all alpha carbons (C2) of even numbered residues were assigned the D-configuration as in "unnatural" D-amino acids. PENAM Version II was then energy minimized as a single strand, in beta-sheet-like
35   extended conformation, using force field parameters offered by the SYBYL software package.

48

As discussed above, it is believed that for optimal binding the PENAM should have a conformation in which the nucleotidic bases are stacked approximately at the 3.5 Angstrom interval and are oriented away from the backbone

5   in such a manner that the hydrogen bond donors and acceptors of the nucleotidic bases can directly "read" the complementary bases on the target nucleic acids by specific hydrogen bonding interactions. The differences can be visualized most readily when the PENAMs are viewed

10 in beta-sheet-like extended conformation. Figure 2a presents a "side view" illustration of the two versions. The differences in estimated orientation are even more striking when viewed from the "top" (i.e. along the length of the polymer) as illustrated in Figure 2b. In

15 particular, in Version I, the adenine moieties in the side chains were approximately 180° apart on the alpha carbons of each pair of adjacent monomers and, as a result, the adenine moieties could not be effectively stacked or aligned at the interval of 3.5 Angstrom

20 spatial distance. In contrast, in PENAM Version II, the adenine moieties were much closer to each other in space (at an angle of approximately 65°) and could therefore be stacked at a spatial distance of approximately 3.7 Angstrom, which substantially enhances their ability to

25 hybridize with a target nucleic acid.

Similar molecular models were constructed using other nucleic bases (e.g., B = thymine, guanine, cytosine, uracil), and confirmed the preceding results.


30   Example 2
Use of molecular modelling to predict homomorphism
between PENAMs of the present invention and target DNA
As an illustration of the use of molecular modelling
to assess the likely homomorphism between a particular

35 version of the PENAMs and a target nucleic acid, we used
the molecular modelling techniques referred to in Example
1 to assess several types of PENAMs having varying spacer

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groups. In particular, we examined models of a single
strand of DNA (a thymine 12-mer) in association with
complementary PENAMs having NuAA monomers of the
following formula:

5

$$
\begin{array}{ccc}
 & & B \\
 & & | \\
 & W & S3 \\
 & | & | \\
\text{—N—S1—E—S2—C—} \\
 & | & \| \\
 & Y & X
\end{array}
$$
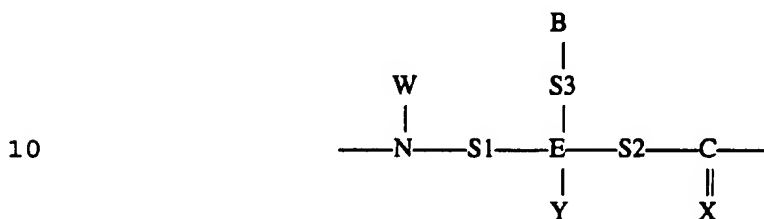
.15   in which E is carbon; W is hydrogen; Y is hydrogen;
S1 is a bond; S3 is an ethylene group; X is oxygen; B is
adenine; N is nitrogen; and wherein S2 was selected from
the group consisting of a bond, a methylene group, an
ethylene group and a propylene group.

20   Energy minimization (100 cycles) was performed using
Tripos force field parameters provided in the Sybyl
software package. Following the teachings of the present
invention, the energy minimization was carried out in a
manner that allowed for the introduction of varying
25   chiral centers in the peptidic backbone of the PENAM such
that the overall energy state of the molecule was
minimized.

The resulting energy-minimized models are shown in
Figure 3(a-d). Although all of the models exhibited the
30   formation of double helices between the PENAM and the
target DNA, the modelling predicted an increasing
homomorphism (which appears as an increasingly regular
helicity) when the S2 spacer group was increased in
length from a bond (Figure 3a), to a methylene group
35   (Figure 3b), to an ethylene group (Figure 3c), and
finally to a propylene group (Figure 3d). The
corresponding model for an interaction between two
complementary strands of DNA is shown in Figure 3e.
While the increase from methylene to ethylene to

propylene is believed to be advantageous, further increasing the length of the S2 group (beyond that of a three-atom bridge as in propylene) is believed to be less advantageous.

5   Additional estimates of the relative homomorphism were obtained by assessing the total energy for the resulting molecules. The total energy was estimated as a sum of bond stretching energy, angle bending energy, torsional energy, out of plane bending energy and van der

10 Waals energy, using the same molecular modelling software. Consistent with the apparently enhanced helicity, as described above, the resulting total energy estimates revealed a considerable decrease in total energy (suggesting an increase in homomorphism) when the

15 length of the S2 spacer group was increased from 0 (in the case of a bond), to 1 (methylene), to 2 (ethylene), to 3 (propylene).

    In all cases, the energy-minimized structures incorporated D-chiral centers into the peptidic backbone.

20 For example, for the particular PENAMs modeled in this illustration, the following D-containing sequences were obtained after 100 cycles of energy minimization: DLLLDDDDDLL in _Figure 3a_; LDDDDDDDDDDD in _Figure 3b_; DDDDDDDDDDDD in _Figure 3c_; and DDDDDDDDDDDD in _Figure 3d_.

25 These modeling data, as well as physical data involving PENAMs with alternating D- and L-chiral centers described below, suggest that the ability to incorporate D-chiral centers into the PENAM can be used to substantially enhance the homomorphism between particular PENAMs and

30 their target nucleic acids.

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Example 3
Use of retrosynthesis and molecular modelling to identify synthetic schemes for the preparation of NuAA monomers

The NuAA monomers of the present invention can be
5   synthesized from available starting compounds, such as
amino acids, via a number of different synthetic
pathways. As described herein, the preferred NuAA
monomers contain a nucleic base that is substituted on
the side chain at a distance which is similar to that
10   between the nucleic base and the sugar-phosphate backbone
in standard ODNs. Molecular modelling and retrosynthesis
techniques   have   been   used   to   identify
conveniently-prepared intermediates and corresponding
synthetic steps for the production of NuAA monomers of
15   the   present   invention.   An   example   of   such   a
retrosynthetic analysis has been illustrated in Figure
4a, as is described in more detail below.

We   have   used   these   techniques   to   develop   a
convergent modular synthetic approach for the synthesis
20   of these stereo-specific PENAMs via NuAA monomers. This
strategy includes a basic set of chemical building blocks
of the structural units which allow for the synthesis of
a diverse class of novel chemotherapeutic agents in an
efficient and cost effective manner.   The chemistry
25   involved in the synthesis of these key intermediates is
fairly easy, extensively studied and is well established
in the literature [63-65, 278, 279].   As described
herein, the preferred building blocks include: (i) NuAA
monomers and their derivatives; (ii) amino acids (AA) and
30   their derivatives; and (iii) aza-amino acids and their
derivatives.

Example 4

Use of α-amino-α-carboxylic acids as substrates for the preparation of NuAA Monomers

As defined earlier, NuAA monomers are amino acids
that have purine/pyrimidine nucleic bases or their
heterocyclic analogs substituted in the aminoacyl side
chain depending from the alpha carbon. These key
intermediates can have L- or D-configurations at the
alpha carbon (except for the aza-NuAA monomers, in which
case the α-carbon is replaced by nitrogen, as described
herein). The distance between the nucleic base and the
peptidic backbone can be easily manipulated by varying
the length of the side chain of these nucleicamino acids,
i.e., the S3 position in the formula of the NuAA monomer.
Molecular modelling has been used to show that, in order
to mimic the homomorphism of native nucleic acids, the
nucleic base is preferably sustituted on the side chain
at least two carbon units distance from the peptidic
backbone (e.g., γ-substituted), and is preferably not
more than four carbon units away from the backbone.
Thus, S3 is preferably a spacer group with a backbone of
at least 2 atoms, preferably at least three atoms. Such
NuAA monomers can be easily synthesized from commercially
available starting materials, for example, natural
alpha-amino acids (an illustrative example of the
retrosynthesis of PENAMs and NuAA monomers from α-amino-
α-carboxylic acids is shown in Figure 4a). These natural
amino acids provide a rich source of diverse chemical
functionality, which are convenient for suitable chemical
transformations using well-known synthetic techniques;
and, in addition, they also serve as chiral templates by
virtue of the chiral center generally present at the
alpha-carbon. As discussed herein, the use of chiral
monomers to introduce varying centers of chirality (or
"antipodes") into the peptidic backbone allows for the
production of PENAMs that are homomorphic to their target

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nucleic acids, allowing for efficient stacking and hydrogen bonding.

The purine/pyrimidine nucleic bases or their heterocyclic analogs can be introduced as aliphatic, acyl, amide or amine side chain substituents by chemical manipulations of these alpha-amino acids (as described herein). Especially convenient precursors for such manipulations are "omega"-halogenated (i.e. terminally halogenated) amino acids. Thus, as illustrated in <u>Figure</u> <u>4a</u>, NuAA monomers can be readily synthesized from ω-halogenated amino acids and any nucleic base. These ω-substituted haloamino acids can themselves be easily synthesized from commercially available natural amino acids, as described below.

Example 5
Preparation of haloamino acids (XAAs)

Based on the retrosynthetic analysis illustrated above, haloamino acids (XAAs) have been identified as convenient chemical building blocks in the synthesis of NuAA monomers. These haloamino acids are modified amino acids in which at least one of the hydrogens on the side chain carbons has been substituted by a halogen group (especially Cl, Br, I). The side chain distance can be readily varied by substituting the halogen group at, e.g., the β-, γ-, δ- or ω- carbon in the side chain. Subsequent nucleophilic substitution of the halogen group by a nucleic base gives ready access to a wide variety of NuAA monomers, which can then be used to prepare stereo specific α-amide linked PENAMs using synthetic techniques analogous to those described in, e.g., references [21-25]. The molecular modelling analyses discussed above suggested that NuAA monomers in which the nucleic base is either at γ (C3) or δ (C4) carbon atoms in the side chain are likely to position and orient the nucleic base at particularly effective distances from the peptidic backbone.

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Although any halo derivative, including chloro-, bromo- or iodo-amino acids is suitable for synthesis of the NuAA monomers, bromo-derivatives were selected as an initial example because there are a number of well-known
5   synthetic routes available for the preparation of brominated amino acids [278, 279]. Of those routes, the conversion of ω-hydroxyamino acids to corresponding ω-bromoamino acids appeared to be especially convenient. In particular, several brominating reagents such as
10 hydrobromic acid, phosphorous tribromide, phosphorous pentabromide, thionyl bromide, methanesulfonybromide and carbon tetrabromide/tertiary organophosphine have been used effectively for the bromination of hydroxy compounds (primary and secondary hydroxyl groups) to bromides [278,
15 279]. The use of carbon tetrahalide-organophosphine is advantageous for converting primary hydroxyl groups to corresponding halides [280]. An illustrative retrosynthetic scheme is shown in Figure 4b.

As will be appreciated by those of skill in the art,
20 advantageous reagents are characterized by simplicity of experimental procedure, good yields, and relatively mild, essentially neutral reaction conditions. In addition, a number of these hydroxyamino acids are commercially available or can be easily prepared in large quantities
25 from their corresponding dicarboxylic esters by reduction with common reducing agents [278, 284]. Accordingly, γ - b r o m i n a t e d - α - a m i n o b u t y r i c   a c i d (4-bromo-2-amino-butanoic acid) and its homologue, δ - b r o m i n a t e d - α - a m i n o - v a l e r i c   a c i d
30 (5-halo-2-amino-pentanoic acid) were identified as particularly convenient intermediates. By way of illustration, the preparation of 4-bromo-2-amino-butanoic acid is described below.

In addition to the halogenation of hydroxy
35 compounds, halogenation of ω-lactones is another useful synthetic methodology for the preparation of halogenated

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carboxylic acids and their derivatives [22-25, 279, 281, 282].

Example 6
5 Synthesis of 4-bromo-2-amino-butanoic acid

For use in the synthesis of NuAA monomers, we prepared enantiomerically pure 4-bromo-2-amino-butanoic acid as L-, D-, and racemic D/L- stereoisomers, suitably protected at their amino and carboxylic acid functional
10 groups in order to facilitate their use in existing solution and solid phase peptide synthesis procedures. As described earlier, 4-bromo-2-amino-butanoic acid can be conveniently prepared from an optically active 4-hydroxy-2-amino-butanoic acid (homoserine) by carbon
15 tetrabromide-triphenylphosphine.   However,   if   the starting material   homoserine   is not   appropriately protected at carboxyl and amino groups, then the chemical conversion   can   lead   to   a   cyclized   product (α-amino-γ-butyrolactone)   instead   of
20 4-bromo-2-amino-butanoic acid. In addition, protection of the α-amino and α-carboxyl groups of homoserine can be affected by its tendency to lactonize. This problem can be readily circumvented by using α-amino-γ-butyrolactone as the starting material. The racemic and L-isomer of
25 4-bromo-2-amino-butanoic acid have been prepared as hydrogen bromide salts in good yield and high optical purity from their corresponding α-amino-γ-butyrolactones by bromination with hydrogen bromide in acetic acid [281].   Although α-amino-γ-butyrolactone is readily
30 available only as a racemate, α-amino-γ-butyrolactone can be synthesized in all stereoisomeric forms either from the corresponding homoserine (γ-hydroxy-α-amino-butanoic acids) or from the corresponding methionine [283]. Even though both D- and L-homoserine are commercially
35 available, their costs currently make them less desirable as starting materials.   Alternatively, D- and L-homoserine can be easily synthesized from readily

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available D- and L- aspartic acids, respectively.
Reduction of the β-carboxyl group of appropriately
protected D- and L- aspartic acid can provide
corresponding protected homoserine derivatives in gram
5 quantities [284], which in turn can be cyclized in the
presence of mineral acid (hydrochloric acid) to yield the
required lactones.

    We used methionine instead of aspartic acid as the
starting material for the synthesis of
10 α-amino-γ-butyrolactone since the D/L-, D- and L-
stereoisomers of methionine are readily available as
inexpensive chiral amino acids and can be converted in
one step to the corresponding D/L-, D-, and
L-α-amino-γ-butyrolactones in high enantiomeric purity
15 and in excellent yields [283].

    All the three stereo isomers, D/L-, D-, and
L-α-amino-γ-butyrolactones were synthesized in about 85%
yield, by modifying the literature procedure of Natelson
[283], as described below.
20

Example 7
Synthesis of the NuAA monomer 4-(N9-adeninyl)-
2-(t-butyloxycarbonylamino)-butanoic acid

    As an illustration of the synthetic procedures, we
25 synthesized D/L-, L- and D- stereo isomers of the NuAA
monomer, 4-(N9-Adeninyl)-2-(t-butyloxycarbonylamino)-
butyric acid [(D/L)-AA(Boc)GAdBA = AdhAla], in good yield
and high optical purity (i.e., greater than 99%), using
D/L-, L- and D- methionine as the chiral precursors,
30 respectively. The synthetic procedure is illustrated in
Figure 5, showing the synthesis of racemic NuAA monomers
[(D/L)-AA(Boc)GAdBA = AdhAla] using D/L-methionine as the
starting material. The structures of the various
intermediates were confirmed using standard techniques
35 including ¹H NMR, ¹³C NMR, specific rotation and mass
spectrometry.

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To summarize this illustrative synthetic procedure, D/L-methionine, on alkylation at the methylmercapto group with bromomethylacetic acid or its ester, formed corresponding sulfonium salts in situ, which on heating

5   underwent nucleophilic attack by water to eliminate methanethiomethylacetate to give corresponding homoserine in situ. This homoserine on further heating at high temperature (>90°C) cyclized to yield the known intermediate α-amino-γ-butyrolactone hydrobromide salt

10 (AAGBL●HBr) in about 85-90% yield. By following the literature procedure [282], this lactone, on treatment with 35% HBr-HOAc at about 75 degrees Celsius, gave corresponding γ-bromoamino acid hydrobromide salt (AAGBBA●HBr). The α-amino group and the α-carboxylic

15 group need to be protected to prevent them from reacting with the γ-bromo group and causing any side reactions during the subsequent synthesis. The α-carboxylic group of γ-bromoamino acid●HBr was protected as its methyl ester (AAGBMB●HCl) by passing dry HCl gas into a

20 methanolic solution, while the α-amino group was protected as a carbamate by treating the methyl ester●HCl with t-butyloxy carbonate in the presence of aqueous NaHCO$_3$ to yield 4-bromo-2-(t-butyloxycarbonylamino)-methylbutyrate, [AA(Boc)GBMB]. The NuAA monomer

25 (D/L)-4-(N^9-adeninyl)-2-amino(^{t}Boc)-butanoic   acid [(D/L)-AA(Boc)GAdBA = AdhAla] was prepared essentially in accordance with these known synthetic procedures [21]. The reaction of this bromo carbamate [AA(Boc)GBMB] with adenine in the presence of dry potassium carbonate and

30 DMF as a solvent gave approximately 70% yield of fully protected NuAA monomer [(D/L)-AA(Boc)GAdMB].

As shown in Figure 6, NuAA monomers in which the nucleic base is other than adenine (A) can be prepared similarly   by   reacting   4-bromo-2-(t-butyloxy-

35 arbonylamino)-methylbutyrate [AA(Boc)GBMB] with the appropriatly protected nucleic base, such as guanine (G),

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cytosine (C), thymine (T), uracil (U) or their heterocyclic analogs.

The individual chemical reactions sumarized above were carried out essentially as described in the
5   literature. The following detailed description of the individual steps is provided for the further convenience of the reader.

(1) **Synthesis of (D/L)-α-Amino-γ- butyrolactone**
10 **hydrobromide [(D/L)-AAGBL•HBr]**:

(D/L)-α-Amino-γ-butyrolactone   hydrobromide [(D/L)-AAGBL•HBr] was synthesized from (D/L)-methionine by following the modified literature procedure referred to above. To an aqueous ethanolic solution (1:1, 200
15 mL), (D/L)-methionine (0.1M, 14.92g) and methylbromoacetate (0.11M, 17.17g) were added at room temperature. The resulting suspension was heated to 55-60°C in an oil bath until it became a homogeneous solution (about 1.5-2 hrs). It was left at that
20 temperature overnight (12 hrs). After 12 hrs the resulting dark yellow solution was heated at reflux for about 3-5 hrs. The thin layer chromatograph (tlc) (n-BuOH:H₂O:HOAc=4:1:1) of this reaction mixture showed that the reaction had gone to completion. The dark brown
25 solution was cooled to room temperature and evaporated to dryness under vacuum using a water aspirator. To this viscous dark brown residue a mixture of isopropanol and toluene (1:1, 50 mL) was added, and the last traces of water were removed by evaporating the resulting solution
30 to dryness. The precipitated, off white crystalline solid was suspended in cold absolute ethanol (50 mL), filtered off, washed thoroughly with a mixture of cold ethanol and toluene (1:1, 50 mL) and air dried. The filtrate and the washings were combined and re-evaporated
35 to generate a viscous liquid which was stirred with 4M HCl in dioxane (60 mL) at -40°C for 4-6 hrs. It was left in a refrigerator overnight. The second crop of the

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precipitated white crystals were filtered off, washed
thoroughly with cold ethanol and air dried. The overall
yield of the product (D/L)-AAGBL•HBr was about 15-15.5 g.
(80-85%). Analogously, the enantiomers (L)-AAGBL•HBr and
5   (D)-AAGBL•HBr were synthesized from (L)-methionine and
(D)-methionine, respectively, and were purified by
recrystallization from 10% aqueous ethanol in about 75%
yield. The structures of all of the stereoisomers were
confirmed by ^{1}H, ^{13}C NMR, specific rotation and mass
10 spectrometry.

(2) Synthesis of (D/L)-α-Amino-γ-bromo butyric acid
Hydrogen bromide [(D/L)-AAGBBA•HBr]:

(D/L)-AAGBL•HBr (0.05M, 9.101g, Aldrich Chemical
15 Co.) was heated with freshly made 35% HBr-HOAc solution
(10 equiv, 0.5M) in a teflon vessel containing a steel
bomb at 75°C for 3 hrs. The steel bomb was cooled to
room temperature. The reaction mixture had precipitated
and formed white crystals. This slurry was transferred
20 to a flask and the contents were evaporated at 40°C using
a water aspirator vacuum to remove excess HBr and HOAc.
The resultant yellowish white crystalline solid was
suspended in a 1:1 mixture of MeOH and toluene and
evaporated again to remove traces of HOAc. The yellowish
25 white crystalline solid was resuspended in diethylether,
filtered off, washed thoroughly with diethyl ether and
air dried to give white crystalline product in almost
quantitative yield (>95%). The proton NMR of this crude
product showed that about 10-15% of the lactone remained
30 unreacted. The product bromoamino acid, (D/L)-AAGBBA•HBr
could be recrystallized from absolute ethanol but it was
used without any further purification for the next step.
The enantiomers (L)-AAGBBA•HBr and (D)-AAGBBA•HBr
were synthesized from (L)-AAGBL•HBr and (D)-AAGL•HBr,
35 respectively, by following the same procedure and were
recrystallized from absolute ethanol. The structures of

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all of the stereoisomers were confirmed by ^{1}H, ^{13}C NMR, specific rotation and mass spectrometry.

(3)   Synthesis of (D/L)-α-amino-γ-bromomethylbutyrate

5   hydrogen chloride [(D/L)-AAGBMB•HCl]:

The methyl ester (D/L)-AAGBMB•HCl was prepared in quantitative yield by dissolving the bromoamino acid, (D/L)-AAGBBA•HBr (0.05M, 13.15g) in an excess of absolute methanol (5mL/mM) and bubbling dry HCl gas through this

10   methanolic solution for about 2 hrs while maintaining the reaction temperature between 35-40°C. The light yellow solution was evaporated below 40°C using a water aspirator vacuum to remove th excess HCl gas and methanol. The residual yellow liquid was diluted with a

15   mixture of methanol and toluene (1:1, 50mL) and evaporated again to dryness to give a syrupy liquid which on further drying at high vacuum gave ivory-white crystals of bromoamino methyl ester, (D/L)-AAGBMB•HCl, which could be purified by crystallization from

20   CHCl$_3$-diethyl ether mixture. For practical purposes, the product was pure enough for the ensuing step.

The enantiomers (L)-AAGBMB•HBr and (D)-AAGBMB•HBr were synthesized similarly from (L)-AAGBBA•HBr and (D)-AAGBBA•HBr respectively and were recrystallized from

25   absolute ethanol and diethylether as co-solvent. The structures of all of the stereoisomers were confirmed by ^{1}H, ^{13}C NMR, specific rotation and mass spectrometry.

(4)   Synthesis of (D/L)-α-(t-Boc)amino-γ-bromo

30   methylbutyrate [(D/L)-AA(Boc)GBMB]:

The carbamate, (D/L)-AA(Boc)GBMB, was prepared by treating the haloamino ester, (D/L)-AAGBMB•HCl with t-butyloxycarbonate in the presence of NaHCO$_3$. To an aqueous solution of (D/L)-AAGBMB•HCl (0.05M, 11.63g; 15mL

35   H$_2$O), an aqueous solution of NaHCO$_3$ (0.11M, 9.24g, 110 mL H$_2$O) was added at 0°C. After the initial evolution of CO$_2$ gas, a solution of t-butyloxycarbonate (0.055M, 12g) in

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p-dioxane (65 mL) was added. The resulting reaction mixture was vigorously stirred at 0°C for an hour and then warmed up to room temperature gradually. The reaction mixture was stirred at room temperature and was
5   monitored by thin layer chromatography. The reaction went to completion within 4-6 hrs. The precipitated oily liquid (denser than water) was separated and the supernatant was extracted with chloroform. The chloroform extract was combined with the oil liquid and
10 was then thoroughly washed with water. The organic extract after evaporation at room temperature provided a light yellow oil with characteristic odor of tBuOH. The product (D/L)-AA(Boc)GBMB was purified as white crystals in about 85% yield by flash chromatography using a
15 gradient of 7.5-15% ethylacetate (EA) in hexane. It could also be crystallized from diethyl ether-petroleum ether mixture.

The enantiomeric methyl esters, (L)-AA(Boc)GBMB and (D)-AA(Boc)GBMB, were synthesized similarly from
20 (L)-AAGBMB•HBr and (D)-AAGBMB•HBr, respectively, and were recrystallized from a diethyl ether and petroleum ether as co-solvents. The structures of all of the stereoisomers were confirmed by ^{1}H, ^{13}C NMR, specific rotation and mass spectrometry.
25

(5)   Synthesis   of   (D/L)-α-Amino(tBoc)-γ-(N9)-adeninyl methylbutyrate [(D/L)-AA(Boc)GAdMB]:

To an oven-dried flask containing a suspension of adenine (0.025M, 3.378g) in freshly distilled
30 dimethylformamide (DMF) (90mL; 3.5 mL/mM base), anhydrous K$_2$CO$_3$ was added. This mixture was stirred for 15 min at room temperature and (D/L)-AA(Boc)GBMB (0.025M, 7.4g) was added in 25 mL dry DMF under an inert atmosphere. The reaction was monitored by thin layer chromatography and
35 the condensation went to completion by leaving the reaction mixture stirred under inert atm at room temperature over 72 hrs. The reaction mixture was

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filtered off to remove the precipitated salts (KBr,
KHCO₃). The filter cake was thoroughly washed with DMF.
The washings were combined with the filtrate and the
resulting solution was evaporated to dryness to give a
5   slurry containing precipitated solid. This viscous
liquid was diluted with CHCl₃ (50 mL) and was filtered to
remove the solid. The organic filtrate was thoroughly
washed with water, dried over Na₂SO₄ and evaporated to
give a yellowish liquid. The N9-substituted product
10   (D/L)-AA(Boc)GAdMB, was purified by flash chromatography
using 4-7% MeOH-EA solvent system in about 80-85% yield.

The adeninyl carbamate methylester enantiomers,
(L)-AA(Boc)GAdMB and (D)-AA(Boc)GAdMB were synthesized
15   similarly from the corresponding bromocarbamate esters,
(L)-AA(Boc)GBMB and (D)-AA(Boc)GBMB and adenine,
respectively. The structures of all of the stereoisomers
were confirmed by ¹H, ¹³C NMR, specific rotation and mass
spectrometry.
20

(6) Synthesis of (D/L)-α-Amino(tBoc)-γ-(N9)-adeninyl
butyric acid [(D/L)-AA(Boc)GAdBA]:
The hydrolysis of the methyl ester was carried out
to provide the corresponding tBoc-protected amino acid as
25   a building block for the solid phase peptide synthesis.
The methyl ester (D/L)-AA(Boc)GAdMB (0.01M, 3.50g) was
dissolved in aqueous dioxane (1:1), and the clear
solution was chilled to 0° to 5°C. To this cold solution
an aqueous solution of LiOH•H₂O (0.0115 M, 0.5 g) was
30   added. The hydrolysis was complete within 3 hrs. of
stirring the solution below 5°C. The reaction solution
was evaporated on a rotovap below 5°C and the last traces
of water and dioxane were removed azeotropically using
benzene as co-solvent. The white solid that was obtained
35   was redissolved in a minimum amount of aqueous dioxane
and then acidified to pH 4-6 with HCl-dioxane solution.
The precipitated white solid was filtered, washed with

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dioxane and dried under high vacuum. The yield of the nucleic aminoacyl monomer (D/L)-AA(Boc)GAdBA was about 90-95%.

5   The nucleic aminoacyl enantiomers (L)-AA(Boc)GAdBA and (D)-AA(Boc)GAdBA were prepared similarly by hydrolysis of the corresponding methylesters. (L)-AA(Boc)GAdMB and (D)-AA(Boc)GAdMB. The structures of all of the stereoisomers were confirmed by ^{1}H, ^{13}C NMR, specific rotation and mass spectrometry.

10

Example 8

Synthesis of NuAA monomers derived from α-amino-(β-/γ-/δ-),α-dicarboxylic acids:

α-amino-(β-/γ-/δ-), α-dicarboxylic acids offer the
15   unique advantage of forming amide bonds (peptide bonds) either at α-carboxylic group or at β-/γ-/δ- carboxylic group.   As   a   consequence   α-amino-(β-/γ-/δ-), α-dicarboxylic acids can be used to introduce flexibility via β-/γ-/δ- amide linkages in the peptide backbone over
20   α-amino-α-carboxylic acids. The α-carboxylic acid group could be chemically manipulated to a suitable side chain, on which the nucleic base is introduced at a homomorphic distance. Various synthetic transformations known in the art can be utilized to prepare these NuAA monomers
25   [63-65]. As an example, a retrosynthetic strategy has been illustrated in Figure 7.

Example 9

Synthesis of aza-NuAA monomers:
30   Aza-NuAA monomers can be derived from nucleic "aza-amino acids". The nucleic aza-amino acids can be easily prepared from aza-amino acid derivatives, such as semicarbazides (aza-amino acid amides) and carbazic acid (hydrazinecarboxylic acid) esters. Usually the aza-amino
35   acid is relatively unstable unless protected at the carboxylic group, due to the fact that N-carboxylic acids are readily decarboxylated. Various synthetic methods,

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known in the art can be utilized to synthesize these aza-
amino acid derivatives [68]. Preparation of aza-amino
acid derivatives can be based on hydrazine chemistry. In
general, a nucleic base or its analog with suitable
5   linker is condensed with hydrazine or a protected
hydrazine. This alkylated or nucleic base substituted
hydrazine is then treated with an appropriate "carbonyl
donor" such as carbonochloridic esters or carbonic
diesters or haloesters. Such a retrosynthetic strategy
10 is illustrated in <u>Figure 8</u>.

<u>Example 10</u>
<u>Assembly of PENAMs from NuAA monomers via solid phase
peptide synthesis</u>

15   Protected NuAA monomers, as described in Example 7,
can be oligomerized using the t-butyloxycarbamate (tBoc)
strategy on the automated peptide synthesizer to prepare
PENAMs of the desired chain length. To summarize, this
strategy requires deprotection of the methyl ester to
20 free the carboxylic acid. The methyl ester
(D/L)-AA(Boc)GAdMB was hydrolyzed by LiOH to yield t-BOC
protected NuAA monomer, (D/L)-AA(Boc)GAdBA. Similarly,
(L)-4-(N9-adeninyl)-2-amino(tBoc)-butyric   acid,
[(L)-AA(Boc)GAdBA] and (D)-4-(N9-adeninyl)-2-amino(tBoc)-
25 butyric   acid   [(D)-AA(Boc)GAdBA]   have   also   been
synthesized from (L)-methionine and (D)-methionine,
respectively.
        The following is an even more detailed description
of the individual synthetic steps:
30   All three stereoisomers, D/L-, L- and D- of the
unnatural NuAA monomer 4-(N9-Adeninyl)-2-amino-butyric
acid [also abbriviated as <u>GAdAABA</u> or <u>AdhAla</u>] and their
derivatives were synthesized in the laboratory in
enantiomerically pure form, as described in the preceding
35 examples. All other amino acid derivatives were of the
L-configuration unless stated, purchased either from
Applied Biosystems Inc. (ABI) or Bachem Inc. and were

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checked for purity by thin layer chromatography, melting
point, and optical rotation. p-methylbenzhydrylamine
(MBHA)   resin,   (copolystyrene-1%   divinylbenzene,
0.69meq/g, 100-200 mesh) was purchased from Peninsula

5   Laboratory, CA. All solvents were purchased either from
ABI or Burdick and Jackson (distilled in glass grade).
Dimethylformamide (DMF) and disopropylethylamine (DIPEA)
were distilled from ninhydrin, followed by calcium
hydride at reduced pressure and stored over molecular

10 sieves (Linde, 4 Angstrom). Boc deprotection reagent,
trifluoroacetic   acid   (TFA),   and   peptide   coupling
reagents,   dicyclohexylcarbodiimide   (DCC),
1-hydroxybenzotriazole (HOBT) were purchased from ABI and
were used as supplied. 1-Benzotriazolyloxy-

15 tris(dimethylamino)-phosphonium hexafluorophosphate (BOP)
and   O-(benzotriazol-1-yl-)-1,1,3,3-tetramethyluronium
hexafluorophosphate (HBTU) were purchased from Richielu
Chemicals Co., Canada.   1-Hydroxy-7-azabenzotriazole
(HOAT) was either synthesized in the laboratory or was

20 purchased from the Miligen Corporation.

Solid phase peptide synthesis was carried out on an
Applied   Biosystem   Inc.   (ABI)   automated   peptide
synthesizer, model 431A, using standard BOC chemistry
protocols.   In order to obtain the end product as an

25 amide   at   the   C-terminus   after   HF   cleavage,
p-methylbenzhydrylamine (MBHA) resin was used as a
polymeric support to anchor the growing peptide chain.
The peptide synthesizer was operated in a semi-automated
manner and all the cycles were modified according to the

30 scale of the reaction and to facilitate the completion of
each coupling reaction.

The PENAMs were synthesized with L-lysine residues
flanking both the N-terminus and the C-terminus because
they are believed to enhance the solubility of the

35 resulting PENAM in aqueous solutions.

A racemic PENAM [H-Lys-{(D/L)-(AhAla)$_{10}$}-Lys-NH$_2$] was
synthesized from 4-(N9-adeninyl)-2-amino-butyric acid

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[(D/L)-GAdAABA] and L-lysine on a peptide synthesizer on
0.2 mM scale. DCC and HOBT were used as the coupling
reagents to couple the lysine residues whereas BOP and
HOBT were used to couple the nucleic aminoacid monomers.

5   As anticipated, the purification of this racemic
PENAM resulted in the generation of a large number of
diastereomers $(2^{10} = 1024)$, which were isolated as a
mixture by HPLC on C-18 reverse phase column. The
molecular weight of this PENAM was in accordance with its
10 calculated mass as determined by mass spectrometry.

Similarly, the enantiomerically pure PENAMs
[H-Lys-{-(D-AdhAla)-(L-AdhAla)-}$_5$-Lys-NH2]   and
[H-Lys-(L-AdhAla)$_{10}$-Lys-NH2] were synthesized from the
corresponding nucleicamino acids, (D)-4-(N9-adeninyl)-2-
15 amino-butyric acid [(D)-GAdAABA or (D)-γ-adeninyl-α-
amino-butyric acid] and (L)-4-(N9-adeninyl)-2-amino-
butyric acid [(L)-GAdAABA or (L)-γ-adeninyl-α-amino-
butyric acid] and (L)-lysine on 0.2 mM scale, by
following the protocols used for the racemic PENAM,
20 [H-Lys-{(D/L)-(AhAla)$_{10}$}-Lys-NH$_2$]. The only difference was
the use of a new coupling reagent HOAT in combination
with HBTU which is believed to increase the coupling
yield and to minimize racemization at the alpha-carbon
[285].

25

Example 11
Binding of a PENAM to a target nucleic acid

Thermal denaturation of an ordered, native structure
of a nucleic acid disrupts the base-pair stacking and, as
30 a result, the UV absorbance increases. The change in UV
absorbance, known as hypochromicity, is a measure of
base-pairing and base-stacking between the two
complementary strands. The UV absorbance profile as a
function of temperature is called a melting curve and the
35 midpoint of this curve is defined as the melting
temperature, T_m, at which 50% of the double strand is
dissociated into its two single strands. Thus, the

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measurement of UV absorbance melting curves provides qualitative and quantitative structural information about the nucleic acid bound to its complementary ligand. T_m also depends on the concentration of the oligonucleotide

5   and the properties of the solvent (buffer: pH, ionic strength, etc.).

Binding studies were carried out by hybridizing the isolated PENAM described above to its complementary oligonucleotide "dT_{10}", followed by thermal denaturation

10 and measurement of UV absorbance as a function of temperature. The synthetic oligodeoxynucleotide (ODN) dT_{10} and its complementary oligodeoxynucleotide dA_{10} were used as the reference nucleic acids. The samples prepared in phosphate buffer (Na_xPO_4) can precipitate

15 magnasium phosphate at high temperature during the thermal denaturation cycle. This problem was readily avoided by repeating the experiment in 10mM sodium cacodylate buffer (Me_2AsO_2Na) instead of the phosphate buffer. The results are summarized in **TABLE 1**.

20

TABLE 1:

Reference Strand	Complementary Strand		Stoichio-metry				T_m (°C)	
dNu	dNu	pNu					Na_xPO_4	Me_2AsO_2Na
dT_{10}	dA_{10}	-	1	: 1	: 0		28.045	29.41
dT_{10}	-	$KpA_{10}K_m$	1	: 0	: 1		31.26	31.00
dT_{10}	dA_{10}	$KpA_{10}K_m$	1	: 1	: 1		31.41	31.052
dT_{10}.	-	$2KpA_{10}K_m$	1	: 0	: 2		30.8	32.57
dT_{10}	dA_{10}	$2KpA_{10}K_m$	1	: 1	: 2		30.07	32.26
$2dT_{10}$	-	$KpA_{10}K_m$	2	: 0	: 1		-	32.28
-	-	$2KpA_{10}K_m$	0	: 0	: 1		no real transition	no real transition

35

dNu = deoxyoligonucleotide; pNu = nucleic oligopeptide;
pA_{10} = PENAM A10; K_m = lysine amide at the C-terminus.

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These data confirm that the PENAM binds to its complementary nucleic acid strand, and that it exhibits a real transition from an ordered structure to a

5   disordered one on thermal denaturation.

Strikingly, the PENAM was observed to melt at an even higher temperature than the reference ODN, $dT_{10}:dA_{10}$. Although the increase in melting temperature was somewhat modest, it is a significant result because it shows that,

10   in spite of being a complex diastereomeric mixture, the PENAMs recognized and bound quite effectively to their complementary oligodeoxynucleotide strands. It is thus expected that certain stereospecific PENAMs will bind to their complementary oligonucleotide targets even more

15   tightly than the observed average for the mixture.

In addition, the binding affinity of these PENAMs can be further enhanced by introducing modified nucleic bases which can improve base pairing by forming additional hydrogen bonds or by conjugation with known

20   nucleic acid intercalating agents such as acridines, anthraquinones, phenanthridines, phenazines, as described above.

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Claims

1. A composition useful in targeting a nucleic acid comprising a stereochemically-selected population of
5 peptide-based nucleic acid mimics (PENAMs) each member of which comprises a sequence of at least about 4 NuAA monomers,

wherein at least one of said PENAMs is a homomorphically-preferred PENAM comprising a D-chiral
10 center or a quasi-chiral and wherein said homomorphically-preferred PENAM makes up at least about 10% of the stereochemically-selected population of PENAMs.

15 2. A PENAM composition of claim 1, wherein said NuAA monomers are each of the following formula:
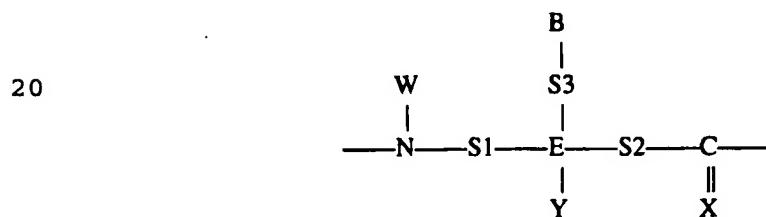
20
```
                        B
                        |
            W           S3
            |           |
         —N——S1——E——S2——C—
                        |        ‖
                        Y        X
```

25

wherein:

E is carbon (C) or nitrogen (N);

W is hydrogen or a spacer group;

Y is hydrogen or a spacer group (when E is carbon),
30 or Y is a lone pair of electrons (when E is nitrogen);

S1 is a bond or a first spacer group;

S2 is a bond or a second spacer group;

S3 is a bond or a third spacer group;

X is oxygen (O) or sulfur (S);
35 B is a base segment comprising a nucleotidic base or an analog thereof; and

N is nitrogen.

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3. A PENAM composition of claim 2, wherein the NuAA monomers in said homomorphically-preferred PENAM are joined directly to each other via peptide bonds and at least one of S1 and S2 in each of the adjacent NuAA
5   monomers is a spacer group.

4. A PENAM composition of claim 2, wherein the NuAA monomers in said homomorphically-preferred PENAM are joined via intervening aminoacyl residues and S2 in each
10   of the NuAA monomers is a bond.

5. A PENAM composition of claim 2, wherein said homomorphically-preferred PENAM comprises a D-chiral center and said D-chiral center is located within a NuAA
15   monomer at position E wherein E is carbon.

6. A PENAM composition of claim 2, wherein said homomorphically-preferred PENAM comprises a D-chiral center and said D-chiral center is located between two
20   NuAA monomers at a chiral carbon in an intervening amino acid residue.

7. A PENAM composition of claim 2, wherein at least about 50% of the chiral centers in said homomorphically-
25   preferred PENAM are D-chiral centers.

8. A PENAM composition of claim 2, wherein about 50% of the chiral centers in said homomorphically-preferred PENAM are D-chiral centers, and 50% of the
30   chiral centers are L-chiral centers, and wherein at least about 80% of L-chiral centers are flanked by D-chiral centers.

9. A PENAM composition of claim 2, wherein at least
35   about 80% of the chiral centers in said homomorphically-preferred PENAM are D-chiral centers.

10. A PENAM composition of claim 2, wherein at least one of S1 and S2 is a spacer group having a backbone of two to three atoms, S3 is a spacer group having a backbone of two to three atoms, W is hydrogen, 5 E is carbon, and Y is hydrogen.

11. A PENAM composition of claim 2, wherein said homomorphically-preferred PENAM comprises a quasi-chiral center and said quasi-chiral center is located within a 10 NuAA monomer at position E wherein E is nitrogen.

12. A PENAM composition of claim 2, wherein said homomorphically-preferred PENAM comprises a quasi-chiral center and said quasi-chiral center is located between 15 two NuAA monomers in an intervening aza-amino acid residue.

13. A PENAM composition of claim 2, wherein all of the NuAA monomers in said homomorphically-preferred PENAM 20 comprise quasi-chiral centers.

14. A PENAM composition of·claim 2, wherein said homomorphically-preferred PENAM further comprises a target modifying group.
25

15. A PENAM composition of claim 14, wherein said target modifying group is located within a NuAA monomer.

16. A PENAM composition of claim 14, wherein said 30 target modifying group is located between NuAA monomers or outside of a string of NuAA monomers.

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17. A free NuAA monomer that is a precursor of a NuAA monomeric subunit comprising a quasi-chiral center wherein said NuAA monomeric subunit comprising a quasi-chiral center is of the following formula:

5

$$
\begin{array}{ccc}
 & & B \\
 & & | \\
W & & S3 \\
| & & | \\
—N—S1—E—S2—C— \\
 & & | \quad\quad \| \\
 & & Y \quad\quad X
\end{array}
$$

10
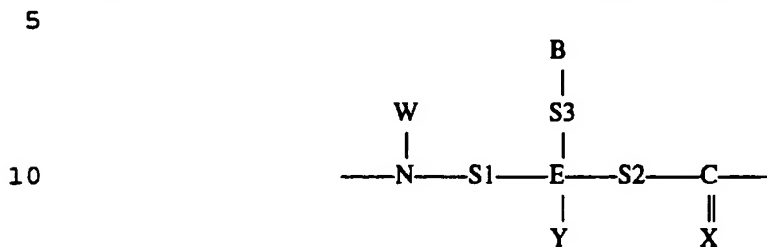
wherein:

15   E is nitrogen (N);

W is hydrogen or a spacer group;

Y is hydrogen or a spacer group (when E is carbon), or Y is a lone pair of electrons (when E is nitrogen);

S1 is a bond or a first spacer group;

20   S2 is a bond or a second spacer group;

S3 is a bond or a third spacer group;

X is oxygen (O) or sulfur (S);

B is a base segment comprising a nucleotidic base or an analog thereof; and

25   N is nitrogen;

wherein said free NuAA monomer is of the same formula except that the N-terminus is in the form of an amine group or a protected amine group and the C-terminus is in the form of a carboxyl group or a protected

30   carboxyl group.

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18.   A free NuAA monomer that is a precursor of a
NuAA monomeric subunit comprising a D-chiral center
wherein said NuAA monomeric subunit comprising a D-chiral
center is of the following formula:

5

```
                          B
                          |
               W          S3
               |          |
   ——N——S1——E——S2——C——
               |          ||
               Y          X
```

10
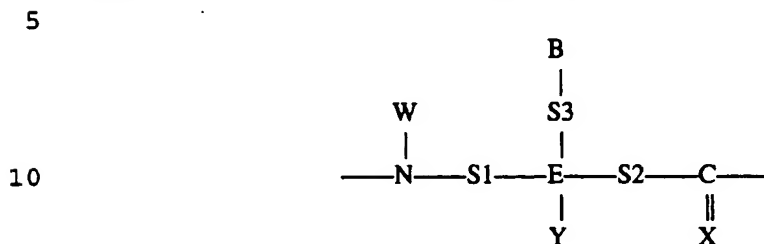
wherein:

15   E is carbon (C);

W is hydrogen or a spacer group;

Y is hydrogen or a spacer group;

S1 is a bond or a first spacer group;

S2 is a bond or a second spacer group;

20   S3 is a third spacer group with a backbone of at
least two atoms;

X is oxygen (O) or sulfur (S);

B is a base segment comprising a nucleotidic base or
an analog thereof; and

25   N is nitrogen;

and wherein at least one of S1 and S2 is a spacer
group, and wherein S3 is a spacer group with a backbone
of at least two atoms;

wherein said free NuAA monomer is of the same
30 formula except that the N-terminus is in the form of an
amine group or a protected amine group and the C-terminus
is in the form of a carboxyl group or a protected
carboxyl group.

35   19.   A method of preparing a PENAM composition of
claim 2 for targeting a target nucleic acid comprising:

(a)   providing at least about 4 NuAA monomers
wherein at least one of said NuAA monomers comprises a D-
chiral center or a quasi-chiral center; and

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(b)   synthesizing an oligomer of the monomers such that the arrangement of bases in the oligomer is substantially complementary to a sequence of nucleotidic bases in a portion of the target nucleic acid.

20.   A method of claim 19, wherein said NuAA monomers are linked to each other directly via peptide bonds.

21.   A method of claim 19, wherein said NuAA monomers are linked to each other indirectly via intervening aminoacyl monomers.

22.   A method of claim 19, further comprising the step of synthesizing a NuAA monomer having a D-chiral center wherein said NuAA monomer having a D-chiral center is present at a purity of at least about 90% enantiomeric excess relative to its enantiomeric NuAA monomer having an L-chiral center.

23.   A method of claim 19, further comprising the step of synthesizing a NuAA monomer having a quasi-chiral center.

24.   A method of targeting a target nucleic acid comprising:
(a) providing a PENAM composition according to claim 2; and
(b)   contacting said PENAM composition with the target nucleic acid.

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25. A method of modulating a target nucleic acid in an antisense manner comprising:

(a) providing a PENAM composition according to claim 2 wherein the sequence of bases in said NuAA monomers is

5   substantially complementary to a sequence of bases in the target nucleic acid; and

(b) contacting said PENAM composition with the target nucleic acid.

10   26. A method of modifying a target nucleic acid comprising:

(a) providing a PENAM composition of claim 2 wherein said homomorphically-preferred PENAM further comprises a target modifying group; and

15   (b) contacting said PENAM composition with the target nucleic acid.

27. A method of detecting a target nucleic acid comprising:

20   (a) providing a PENAM composition according to claim 2 wherein the sequence of bases in said NuAA monomers is substantially complementary to a sequence of bases in the target nucleic acid; and

(b) contacting said PENAM composition with the

25 target nucleic acid; and

(c) detecting a target complex comprising said nucleic acid mimic and said target nucleic acid or detecting a modification in the target nucleic acid.

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28.   A method of isolating a target nucleic acid comprising:

(a) providing a PENAM composition according to claim 2 wherein the sequence of bases in said NuAA monomers is
5   substantially complementary to a sequence of bases in the target nucleic acid; and

(b)   contacting said PENAM composition with the target nucleic acid; and

(c) isolating PENAMs bound to said target nucleic
10   acid or isolating a modified target nucleic acid.

1/14
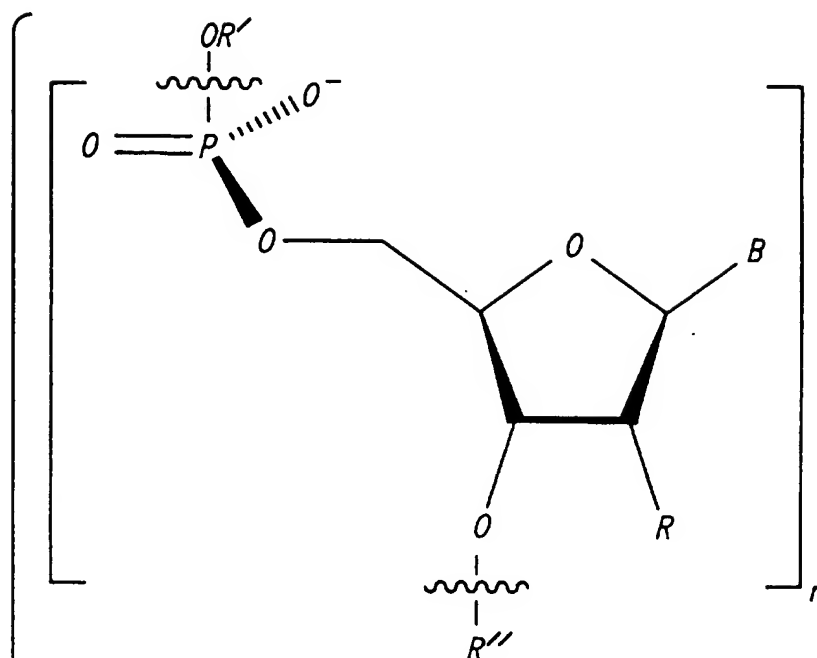
DNA/RNA/ONT
B = A, T, G, C, U
R = H, OH; R′ = R″ = PROTECTIVE GROUPS

FIG. 1
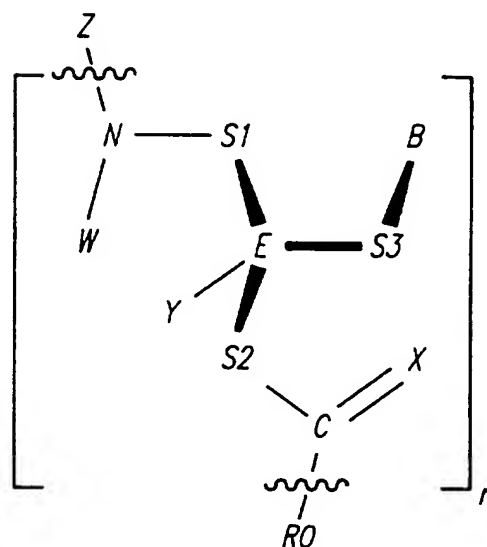
PEPTIDE NUCLEIC ACID MIMIC (PENAM)
E = C, N; X = O, S
B = A, T, G, C, U, & Pu/Py ANALOGS
S1, S2, S3, W, Y = SPACER GROUPS
Z, R = PROTECTIVE GROUPS
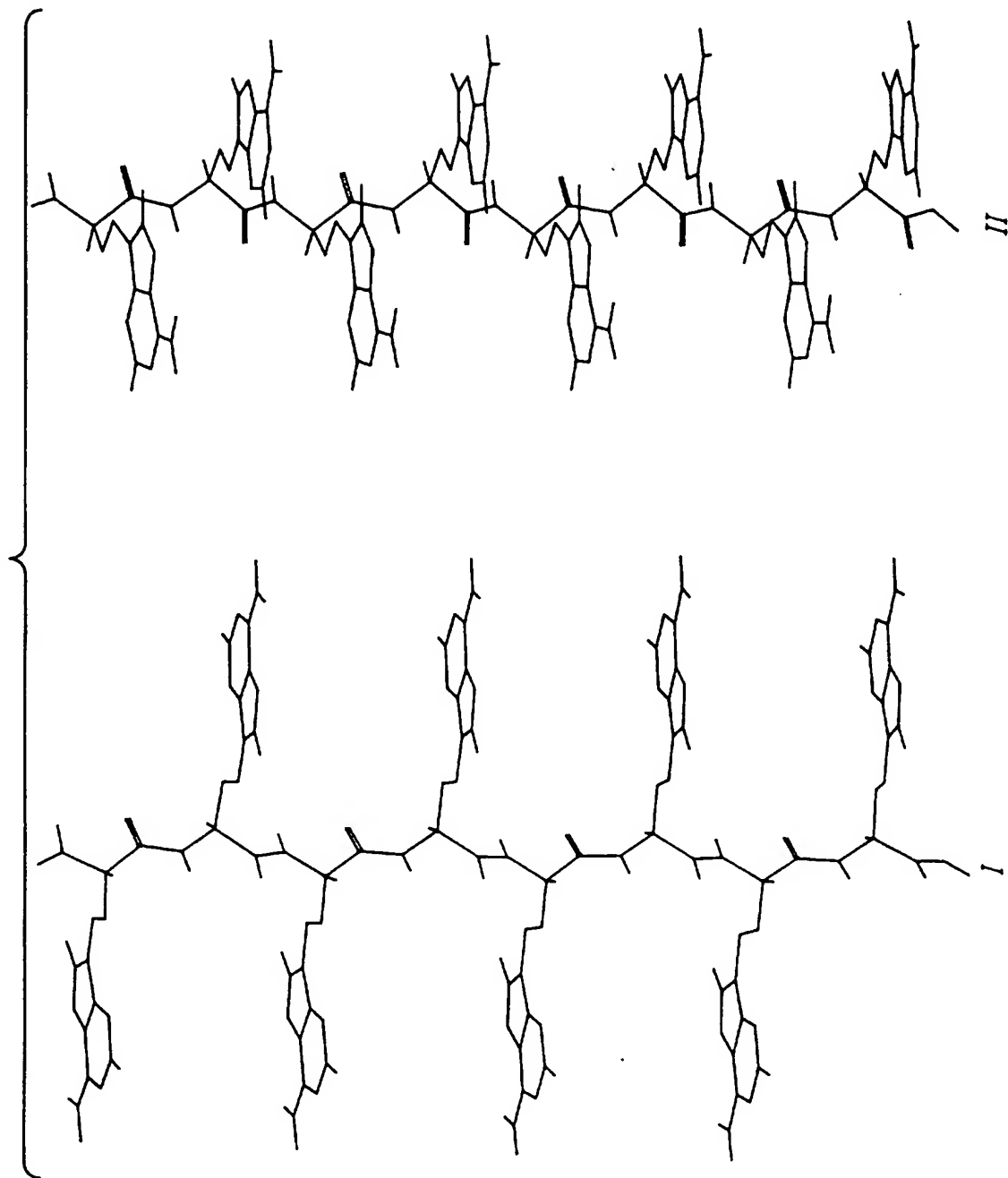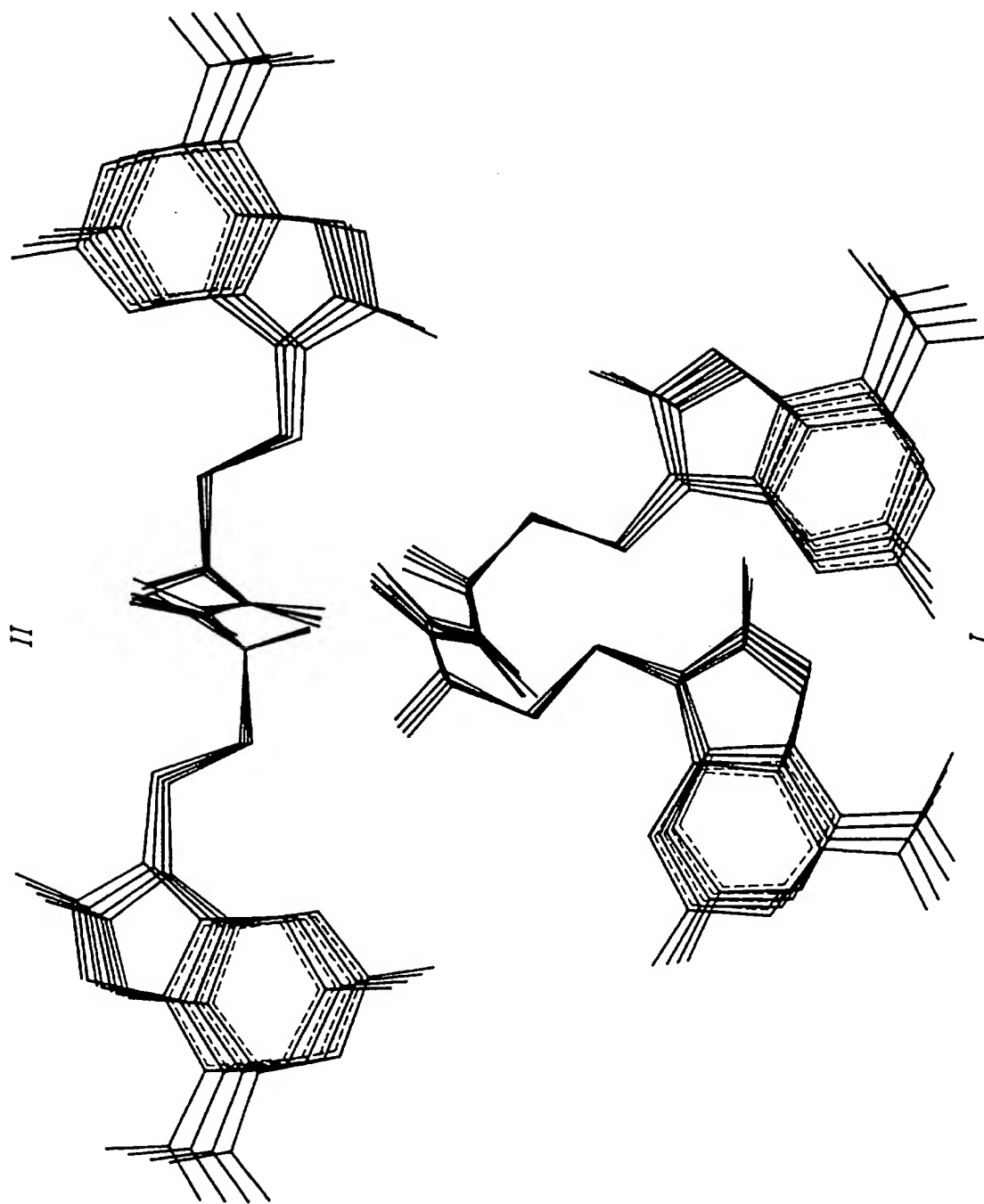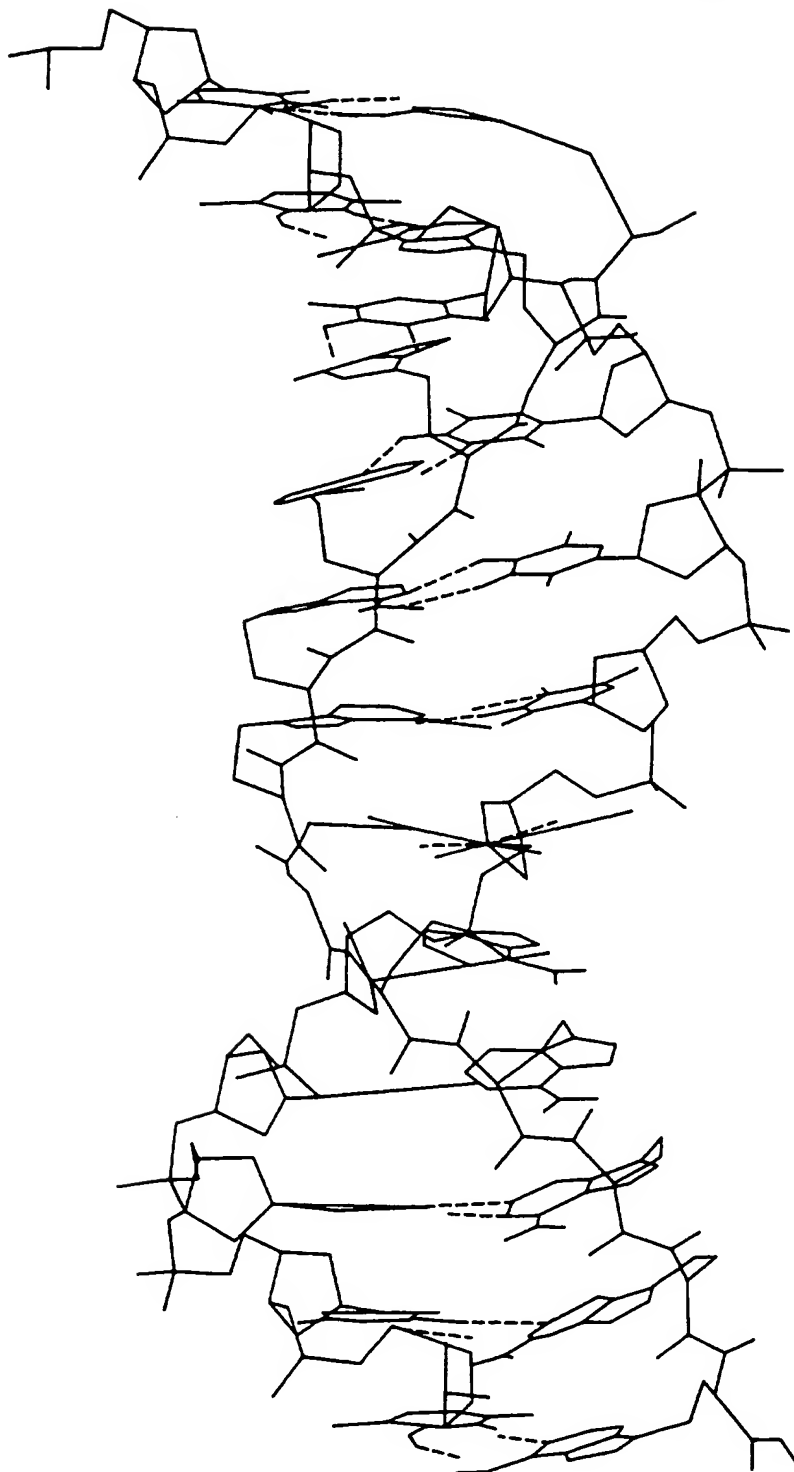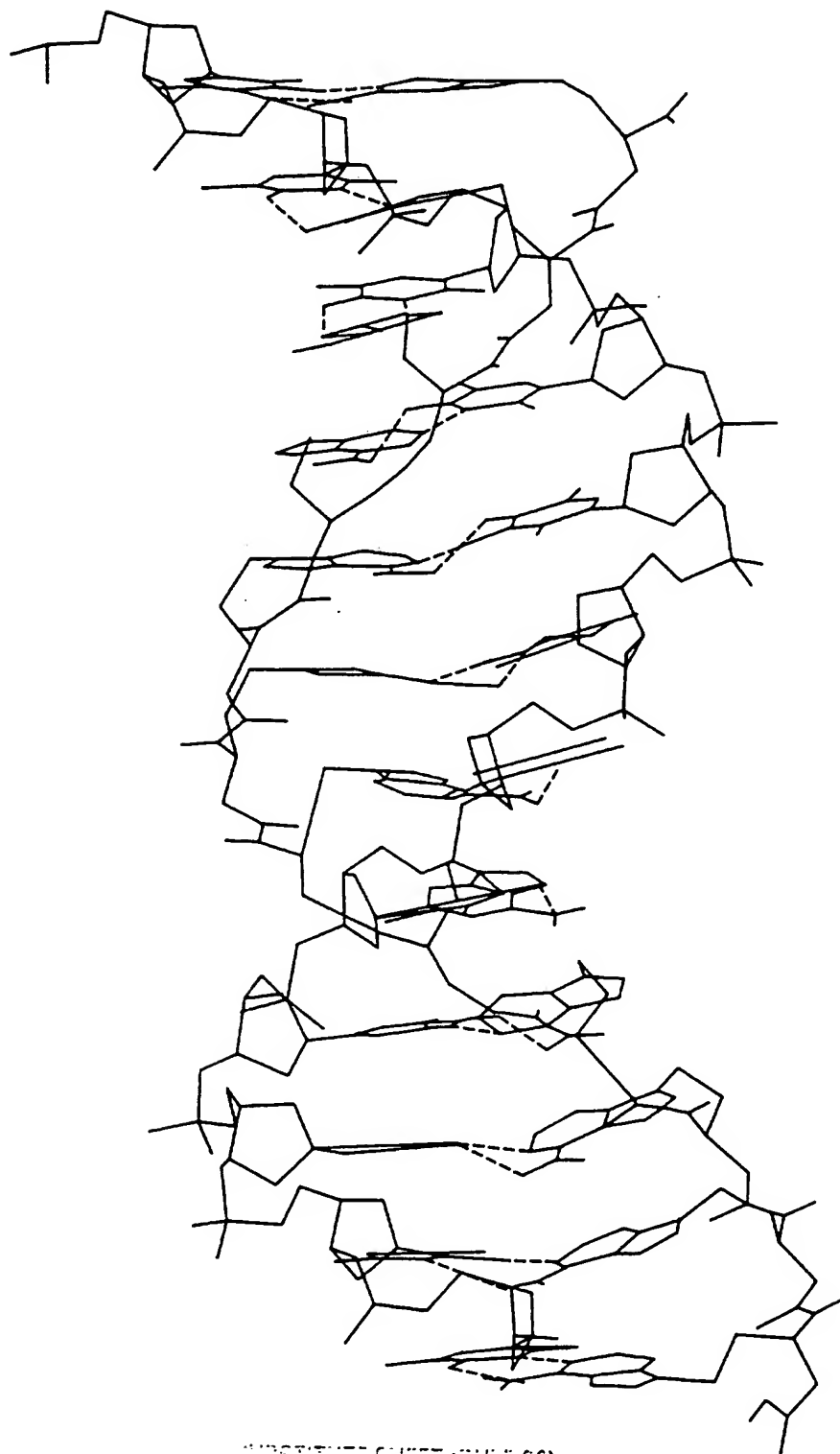
FIG. 2A

FIG. 2B

FIG. 3A

FIG. 3B

FIG. 3C

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FIG. 3D

FIG. 3E

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FIG. 4A

(D/L)-PEPTIDE NUCLEIC ACID MIMIC (PENAM)
Nu = A, T, G, C, U, Pu/Py ANALOGS
Z, R = PROTECTIVE GROUPS; p = e.g. 2 – 3...; n = e.g. 2 – 8...

(D/L)-NUCLEIC AMINOACID (NuAA)
Nu = A, T, G, C, U, Pu/Py ANALOGS
p = e.g. 2 – 3...

(D/L)-HALO AMINOACID (XAA)
X = Br, Cl, I, p = e.g. 2 – 3...

(D/L)-ω-HYDROXY AMINOACID
P = e.g. 1 – 3...

P = 4

(D/L)-HOMOGLUTAMIC ACID

p = 3

P = 1

(D/L)-ASPARTIC ACID

p = 3

(D/L)-α-AMINO-ω-LACTONE
p = e.g. 1, 2, 3...

P = 2   *P = 1*

(D/L)-GLUTAMIC ACID   *(D/L)-METHIONINE*
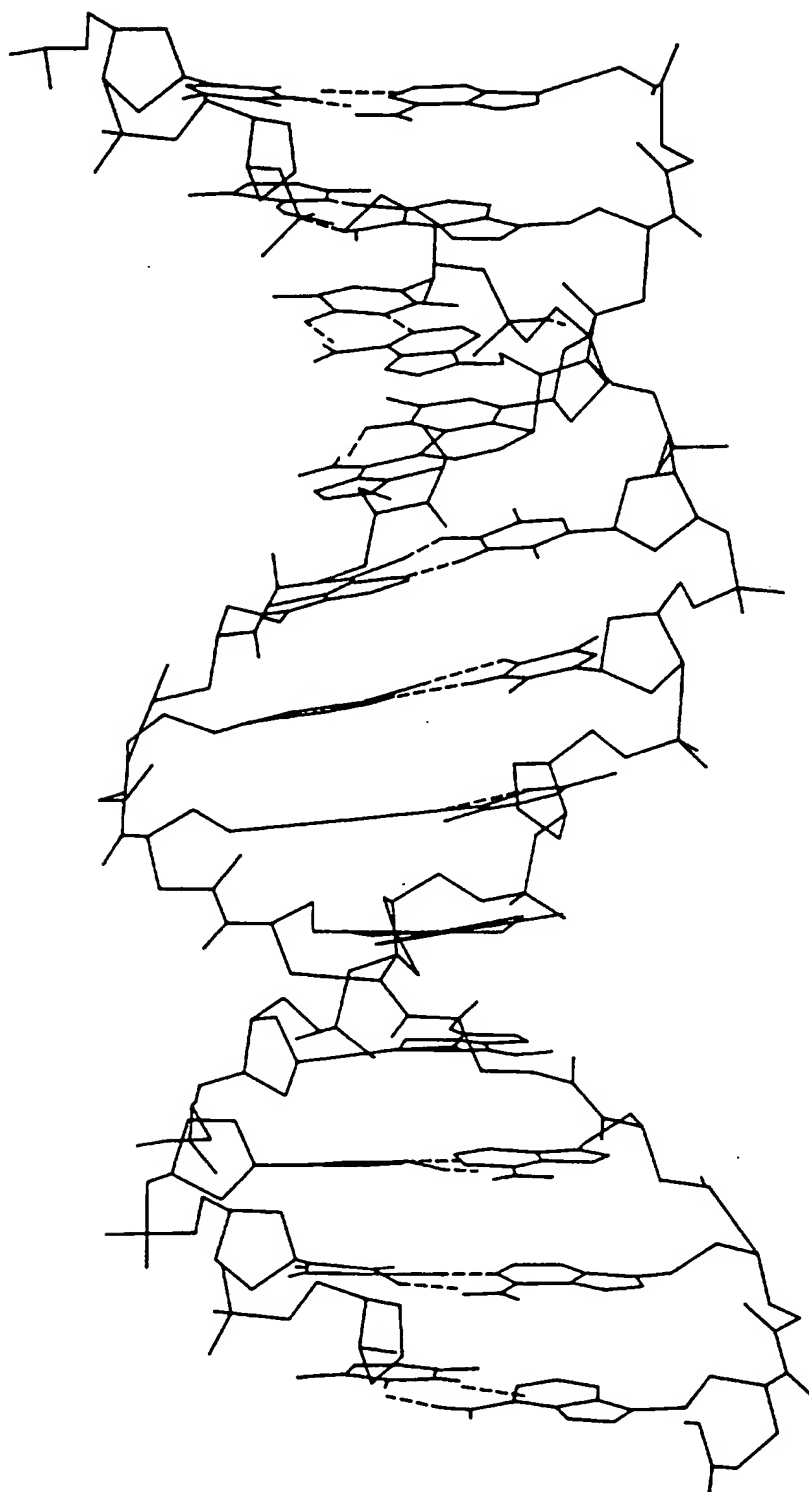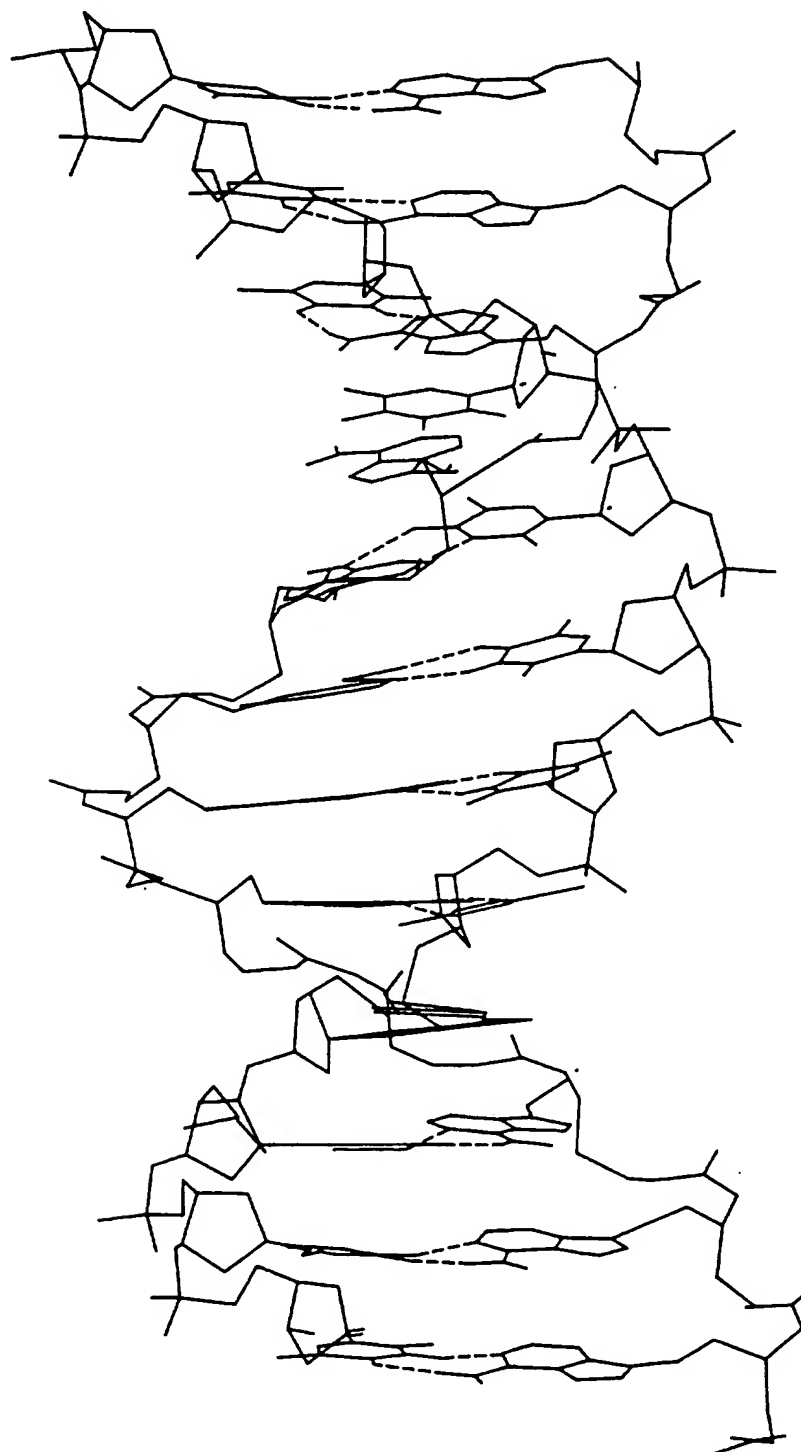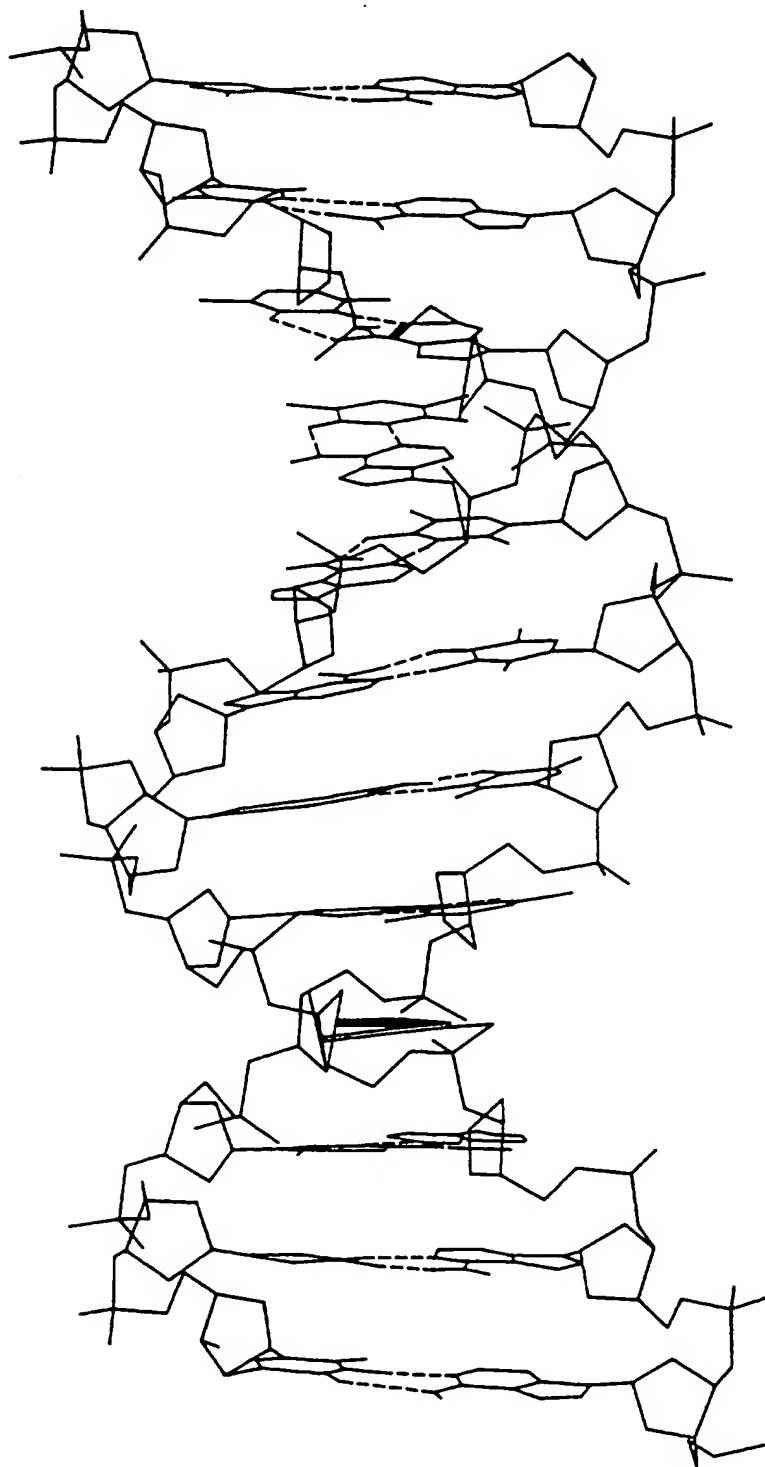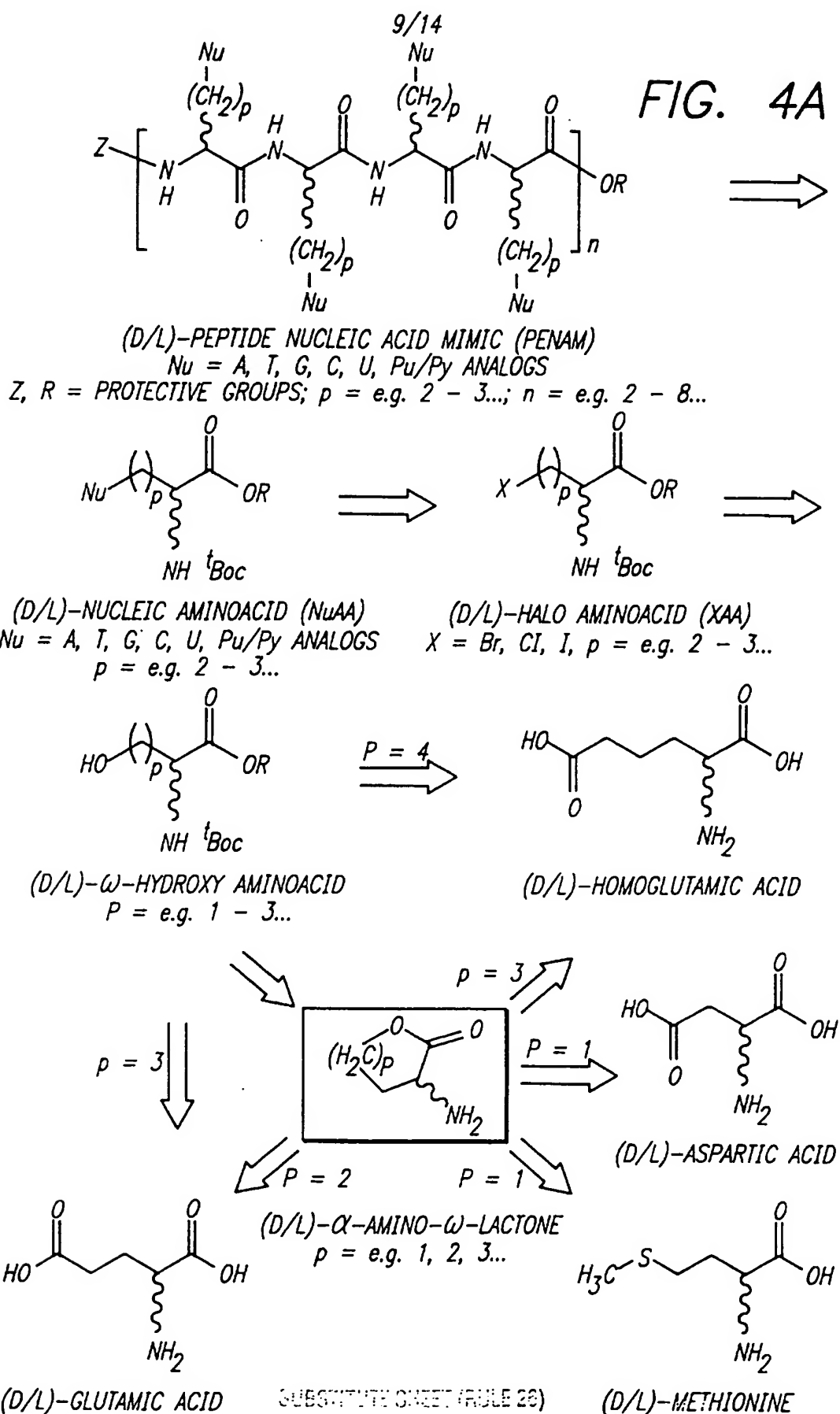
FIG. 4B
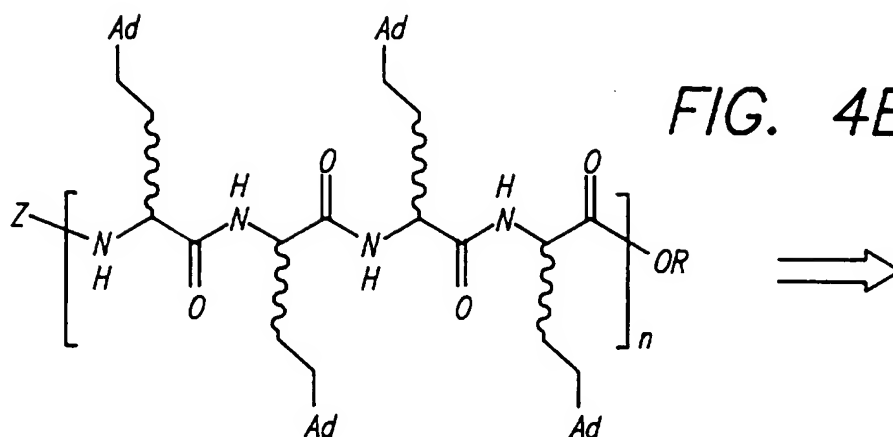
(D/L)-PEPTIDE NUCLEIC ACID MIMIC (PENAM)
Ad = ADENINE
Z, R = PROTECTIVE GROUPS; n = e.g. 2 - 8...
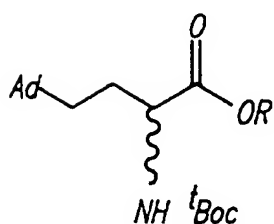
(D/L)-NUCLEICAMINO ACID (AdhAla)
Ad = ADENINE

(D/L)-HALOAMINO ACID (XAA)
X = Br, I, CI

(D/L)-HOMOSERINE

(D/L)-ASPARTIC ACID

(D/L)-α-AMINO-γ-BUTYROLACTONE

(D/L)-METHIONINE

FIG. 5

(D/L)-METHIONINE → (BrCH₂COOMe, Aq. EtOH, 55°C, 12 h) → (D/L)-AAGBL·HBr → (35% HBr-HOAc, 75°C, 4 h)

(D/L)-AAGBBA·HBr → (HClg/MeOH, 35-40°C, 2 h) → (D/L)-AAGBMB·HCl → ((BOC)₂O/NaHCO₃, Aq. DIOXANE, RT, 3 h)

(D/L)-AA(Boc)GBMB → (K₂CO₃/DMF, ADENINE, RT, 72 h) → (D/L)-AA(Boc)GAdMB

(LiOH, Aq. DIOXANE, 5°C, 3 h) → (D/L)-AA(Boc)GAdBA (AdhAla) → ((D/L)-AA(Boc)GAdBA, PEPTIDE SYNTHESIZER)

—[(D/L)-AdhAla-(D/L)-AdhAla]ₙ—   n = 2 - 8...

(D/L)-PEPTIDE NUCLEIC ACID MIMIC (PENAM)

FIG. 6

1. Nu = C, a
2. b

(D/L)-AA(Boc)GCyBA
a = K₂CO₃/DMF, RT, 72h
b = LiOH/aq. DIOXANE

1.Nu = A, a
2. b

(D/L)-AA(Boc)GAdBA
a = K₂CO₃/DMF, RT, 72h
b = LiOH/aq. DIOXANE

(D/L)-AA(Boc)GBMB

1.Nu = U, a
2. b

(D/L)-AA(Boc)GUrBA
a = K₂CO₃/DMF, RT, 72h
b = LiOH/aq. DIOXANE

1. Nu = T, a
2. b

(D/L)-AA(Boc)GThyBA
a = K₂CO₃/DMF, RT, 72h
b = LiOH/aq. DIOXANE

1.Nu = G, a
2. b

(D/L)-AA(Boc)GGuBA
a = K₂CO₃/DMF, RT, 72h
b = LiOH/aq. DIOXANE

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FIG. 7

(D/L)-ω-PEPTIDE NUCLEIC ACID MIMIC (PENAM)
Nu = A, T, G, C, U, Pu/Py ANALOGS
Z, R = PROTECTIVE GROUPS
p = e.g. 1-3... ;
m = e.g. 1-3... ; n = e.g. 2-8...

(D/L)-ω-NuAA
Nu = A, T, G, C, U, Pu/Py ANALOGS
Z, R = PROTECTIVE GROUPS
p = e.g. 1-3... ; m = e.g. 1-3...
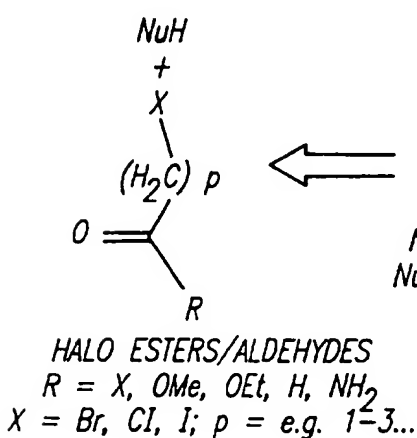
HALO ESTERS/ALDEHYDES
R = X, OMe, OEt, H, NH₂
X = Br, CI, I; p = e.g. 1-3...
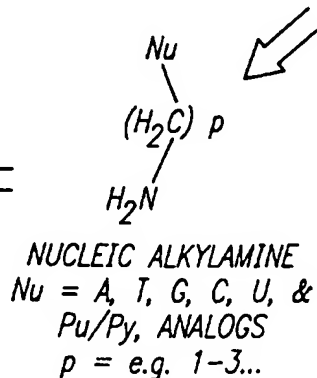
NUCLEIC ALKYLAMINE
Nu = A, T, G, C, U, &
Pu/Py, ANALOGS
p = e.g. 1-3...
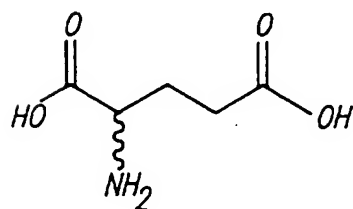
(D/L)-GLUTAMIC ACID
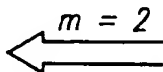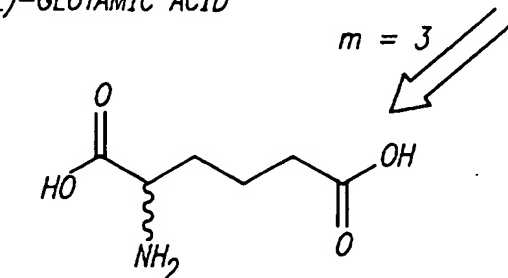
m = 2

(D/L)-α-AMINO-ω,α-DICARBOXYBLIC ACID
m = e.g. 1-3...

m = 3

(D/L)-HOMOGLUTAMIC ACID

m = 1
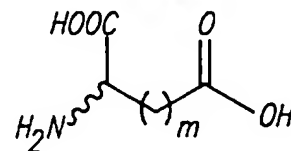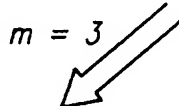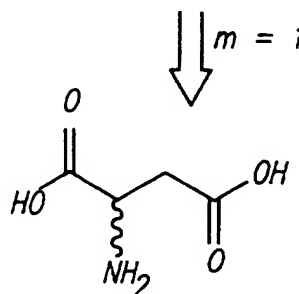
(D/L)-ASPARTIC ACID
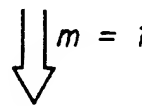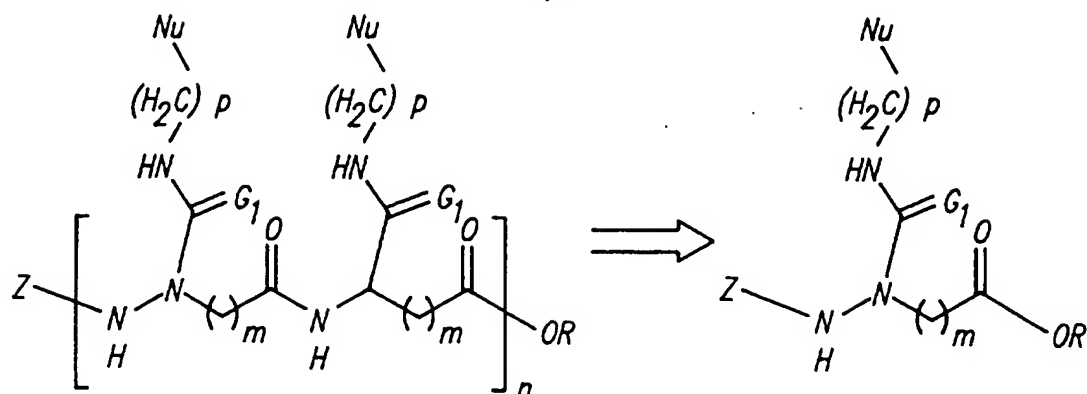
ω-azaPEPTIDE NUCLEIC ACID MIMIC (azaPENAM)
Nu = A, T, G, C, U, Pu/Py ANALOGS
Z, R = PROTECTIVE GROUPS;
G1 = O, S, H2; p = e.g. 1-3... ;
m = e.g. 0-3... ; n = e.g. 2-8...

ω-NuAzaAA
Nu = A, T, G, C, U, Pu/Py ANALOGS
Z, R = PROTECTIVE GROUPS;
G1 = O, S, H2
p = e.g. 1-3... ; m = e.g. 0-3...

NUCLEIC ALKYL DERIVATIVES
G1 = O, S, H2
G2 = OMe, OEt, X, H
p = e.g. 1-3...

HALO DERIVATIVES
G1 = O, S, H2
G2 = OMe, OEt, X, H
X = Br, CI, I; p = e.g. 1-3...

HALO ESTERS
R = Me, Et, H
X = Br, CI, I
m = e.g. 0-3...

α-AZAMINO-ω-ACID
R = Me, Et, H; m = e.g. 0-3...

HYDRAZINE
Z = PROTECTIVE GROUP
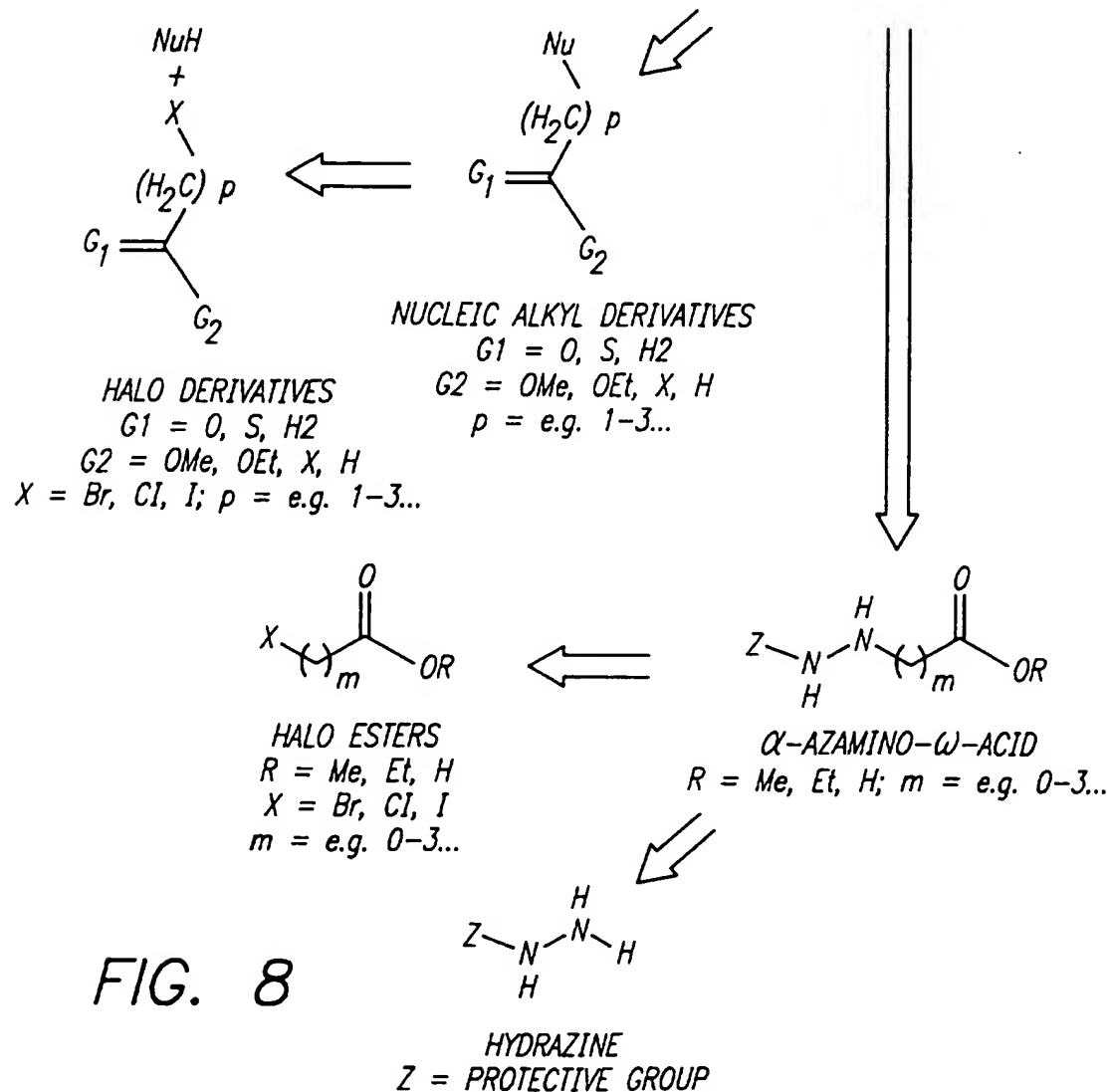
FIG. 8

International application No.

PCT/US95/09828

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :536/23.1, 24.5, 25.4; 530/333; 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 24.5, 25.4; 530/333; 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS, MEDLINE
search terms: oligo?, nucleic acid, nucleotide, purine, pyrimidine, peptide, amino(w)acid, mimic

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of the American Chemical Society, Volume 114, issued 1992, R. Zuckermann et al., "Efficient Method for the Preparation of Peptoids [Oligo(N-Substituted Glycines) by Submonomer Solid-Phase Synthesis", pages 10646-10647, see entire document.	1-25
Y	Nucleic Acids Research, Volume 21, No. 8, issued 1993, D. Ecker et al., "Rational Screening of Oligonucleotide Combinatorial Libraries for Drug Discovery", pages 1853-1856, see entire document.	1-25
Y	Gene, Volume 149, issued 1994, P. Nielsen et al., "Sequence-Specific Transcription Arrest by Peptide Nucleic Acid Bound to the DNA Template Strand", pages 139-145, see entire document.	1-25

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*	Special categories of cited documents:	
"A"	document defining the general state of the art which is not considered to be of particular relevance	
"E"	earlier document published on or after the international filing date	
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O"	document referring to an oral disclosure, use, exhibition or other means	
"P"	document published prior to the international filing date but later than the priority date claimed	

"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 NOVEMBER 1995	20 DEC 1995

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	CHARLES C. P. RORIES
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Advances in Polymer Science, Volume 41, issued 1981, K. Takemoto et al., "Synthetic Nucleic Acid Analogs, Preparation and Interaction", pages 1-51, see entire document.	1-25
Y	Journal of the American Chemical Society, Volume 114, issued 1992, M. Egholm et al., "Peptide Nucleic Acids (PNA). Oligonucleotide Analogues with an Achiral Peptide Backbone", pages 1895-1897, see entire document.	1-25
Y	Science, Volume 354, issued 06 December 1991, P. Nielsen et al., "Sequence-Selective Recognition of DNA by Strand Displacement with a Thymine-Substituted Polyamide", pages 1497-1500, see entire document.	1-25
Y	Science, Volume 258, issued 27 November 1992, J. Hanvey et al., "Antisense and Antigene Properties of Peptide Nucleic Acids", pages 1481-1485, see entire document.	1-25

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-25

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/00; C07D 237/00, 473/00; C07H 21/00; C07K 1/00, 2/00; C12Q 1/68

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-25, drawn to a composition comprising a peptide-based nucleic acid mimic (PENAM), a monomeric precursor of a PENAM, a method for making a PENAM, and a method for using a PENAM as an antisense inhibitor of nucleic acid metabolism, classified in Class 536, subclass 24.5, for example.

II. Claim 26, drawn to a method for modifying the chemical structure of a target nucleic acid, classified in Class 435, subclass 91.1, for example.

III. Claim 27, drawn to a method for detecting a nucleic acid, classified in Class 435, subclass 6, for example.

IV. Claim 28, drawn to a method for isolating a nucleic acid, classified in Class 536, subclass 25.4, for example.

The claims lack unity of invention under 37 C.F.R. 1.475(d), as the claims of Groups II, III, and IV are each drawn to a separate method for using the disclosed antisense nucleic acid mimic that is materially different from the first-to-be claimed method for using the antisense nucleic acid mimic to inhibit nucleic acid metabolism which is included in Group I.